Influence of drought stress on interactions of cotton (Gossypium hirsutum), twospotted spider mites (Tetranychus urticae), and western flower thrips (Frankliniella occidentalis).

by

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Abstract

Drought is a concern for crop production in the High Plains region of the United States which is predicted by climate models to become exacerbated by regional climatic changes and high-output irrigation that is diminishing the finite underground water resources of the Ogallala Aquifer. In addition to drought conditions, changes in pest pressure due to indirect effects of drought stress also occur. The western flower thrips (*Frankliniella occidentalis*) is known as an economically important early-season pest insect pest of cotton and it also preys on the eggs of the herbivorous twospotted spider mite (*Tetranychus urticae*). It is unknown how the relationship between these two arthropod species could be altered during drought conditions in an agricultural setting. Chapter 1 discusses the interplay of these issues and states that the overall goal of the thesis was to examine the net effect of drought on plant-arthropod interactions.

Chapter 2 examined the three-way responses of the plant and two arthropods to drought stress in a controlled greenhouse environment over a two week period. Drought conditions directly reduced spider mite populations, plant development, and stomatal gas exchange. Also, drought had indirect negative effects on plant development and physiological functions from the pestiferous activity of the arthropod populations. We infer that these plant responses reduced the seedling's quality as a host plant which resulted in the observed reductions in spider mite populations when thrips were absent as well as when they were present. The net effect of drought on seedling cotton was compounded by the arthropod dynamics, despite any predation thrips may have enacted on the mite egg populations.

Chapter 3 examined the question: do thrips adjust their omnivorous feeding behavior under drought conditions? We approached this question with a 72-hour experiment in which we recorded the amount of herbivorous and predacious feeding by thrips under well-watered and

drought conditions. Thrips consumed more mite eggs under drought conditions while the amount of thrips feeding scars on the plant remained the same. We suspect that thrips are supplementing their diet with mite eggs because of reduced plant quality and potential plant defense hormones.

Chapter 4 expands on how the results from these two experiments support our conclusion that the net effect of drought stress is largely negative for early-season cotton seedlings. Application of the findings from this thesis can assist with present-day pest and crop management in areas such as proper pesticide utilization and water conservation, which will prepare crop producers for future climatic conditions.

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Chapter 1 - Thesis Introduction and Background

The agricultural industry in the High Plains region of the United States is a major contributor to food security in the United States and globally. However, much of the High Plains region is considered a semi-arid environment, with an average annual precipitation of less than 65 centimeters. Any additional water must come in the form of irrigation, so producers rely on the continent's largest underground aquifer known as the Ogallala Aquifer (shown as the High Plains Aquifer in Figure 1). With the wide-spread adoption of pivot irrigation in the mid-20th century, the depletion rate of this subsurface water supply for crops has nearly doubled and natural water recharge rates are too slow to replenish the amount that is being removed (Konikow 2015). This imbalance presents a threat to the longevity of the water supply and has forced many farmers into pumping fewer gallons per acre due to the dropping well capacities (Buchanan et al. 2001). Additionally, climate models predict a high likelihood of an increase in drought severity for this region (Karl et al. 2009, Basara et al. 2013), which will influence the irrigation output required to sustain profitable crop production. Dry climatic conditions coupled with less water available from the aquifer could lead to additional plant stress. Furthermore, stressed plants often are more attractive to, and more quickly colonized by, pests that can compromise plant health (Ladányl and Horváth 2010). Therefore, it is essential for the future success of agriculture in the High Plains region and the U.S. economy to understand how these challenging conditions will alter the plant-pest dynamics in agroecosystems.

Farmers in the High Plains approach these issues by using new techniques such as selecting crops that remain profitable without a high demand of water. Cotton (*Gossypium hirsutum*) is considered a low water-use crop, which has been grown successfully throughout the southern High Plains for nearly two centuries (White 1931), and the majority of modern U.S.

cotton is produced in this region (National Agricultural Statistics Service 2017). Twospotted spider mites (*Tetranychus urticae*) and western flower thrips (*Frankliniella occidentalis*) are both classified in the phylum Arthropoda and pests of cotton plants that can pose a high threat to young seedlings. The possibility for both drought and arthropod pests to exert stress on cotton provides an opportunity to use cotton as a model crop system for studying the dynamic interactions of water stress and early-season pest infestation, and how those interactions might impact seedling health and development.

Under persistent drought conditions, cotton has traits that allow it to conserve water. Stellate or star-shaped trichomes, thick leaves, a woody stem, and deep roots are morphological properties that reduce heat and transpiration. Additionally, cotton responds physiologically to drought stress by stunting above-ground development and closing stomatal pores (Pace et al. 1999), both of which act to keep water within the plant. However, the plant is not immune to the effects of water loss. A direct result of stomatal closure is an increase in leaf temperature, which can lead to severe desiccation, wilting, and even death of the plant if conditions are severe (Fiene 2012). This increase in temperature from drought can also have indirect effects on the plant by increasing pest population development.

Spider mites and thrips increase egg oviposition rates and offspring development rates at temperatures between 20-30°C (Margolies and Wrensch 1996, Carey and Bradley 1982, Steenbergen et al. 2018). These conditions are often exceeded during times of drought in the summer cropping season. Changes in life cycle development of residing pests mean that the next generation of offspring will arrive sooner than under cooler conditions, which increases the risk of plant damage during vulnerable seedling development. Additionally, environmental conditions and stomatal closure cause lower relative humidity at the leaf surface because

transpiration is reduced. Boudreaux (1958) showed that a 60% lower relative humidity microclimate resulted in higher adult female spider mite oviposition rates. This cascade of changes under intensifying environmental drought stress leads to a more prolific mite population in the field (English-Loeb 1990). Thrips reared on water-stressed plants have been shown to have reduced survival due to the lower humidity at the leaf surface (Shipp and Gillespie 1993). However, thrips are highly mobile in nature and their thigmotactic tendencies, which is to hide in the tight folds of newly emerging leaves, could allow them to escape threatening low-humidity microclimates.

Despite being pests of cotton, western flower thrips also feed on coexisting twospotted spider mite eggs. Conceptually, this predatory behavior sounds beneficial to producers, but these benefits will depend on many factors in the field which determine the relative amounts of herbivory versus predation. Factors such as egg availability and plant quality have been shown to alter the rate of thrips consumption of mite eggs (Agrawal et al. 1999, Coll and Guershon 2002, Trichilo and Leigh 1988), but the effects of drought on thrips omnivory have not been explored. A part of this thesis research aims to help answer that question.

The overall focus of this thesis is to examine the effects of drought on the complex interactions involving plants and arthropods with the goal of gaining a better understanding of the multiple ways that water stress may affect plant stress. Ultimately, the knowledge gained will help cotton producers understand how drought and irrigation regimes impact mite and thrips populations, and to predict if insecticidal treatments will be needed to manage these pests. This knowledge can be used to predict if insecticidal treatments will be needed to manage these pests, which could ultimately save cotton farmers and scouts money and time.

Figures

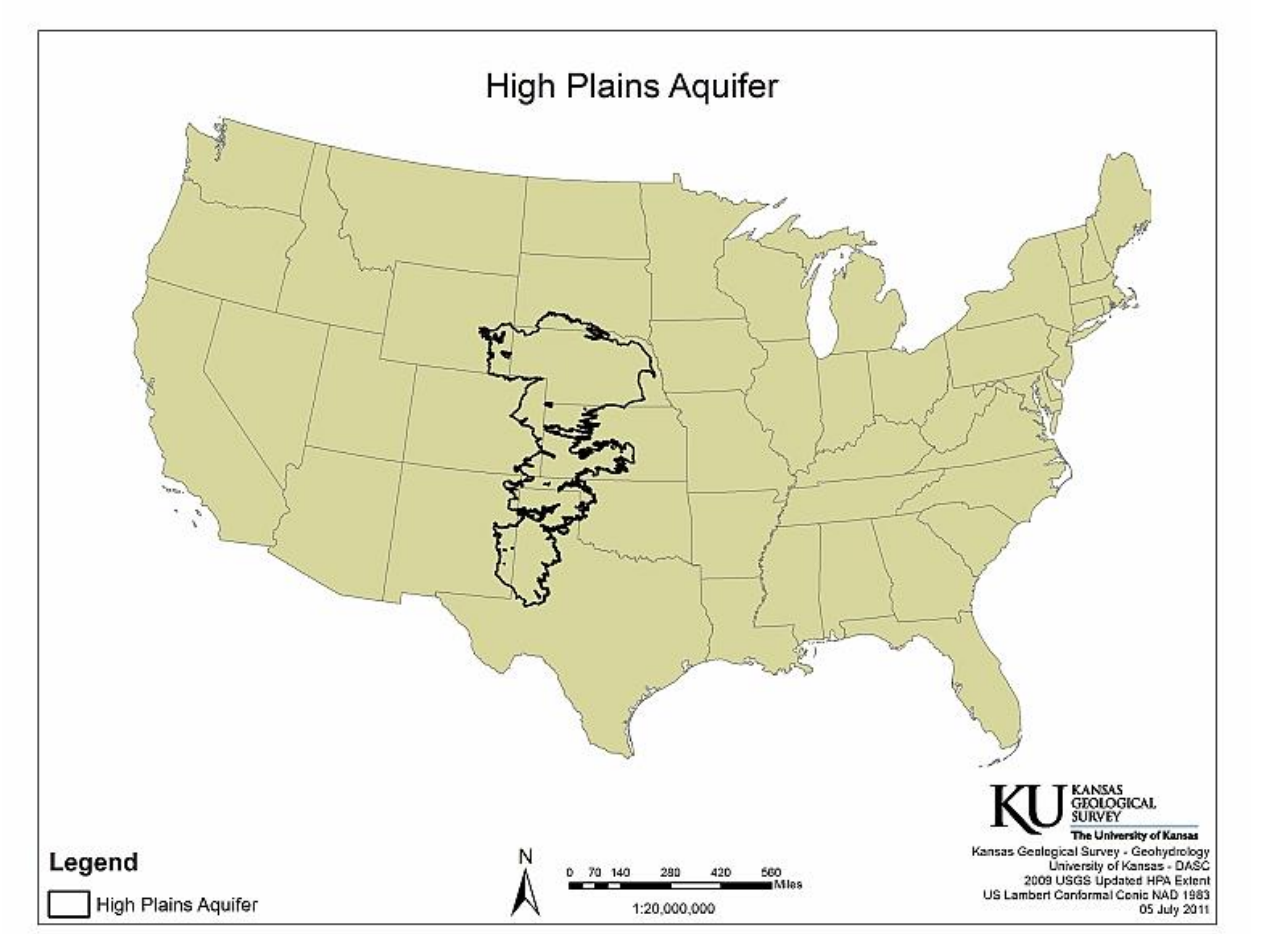

Figure 1.1 Map showing the High Plains Aquifer region of the United States.

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Chapter 2 - Direct and indirect effects of drought on plant-arthropod tri-trophic interactions in early season cotton

Introduction

Crop producers in the High Plains region of the central United States have long been challenged by the inadequate amount of rainfall to sustain crops through the hotter drier conditions than those to the east. Innovative irrigation techniques have their origins here, most of which utilize ground water resources from the expansive Ogallala Aquifer, such as the central pivot irrigator and subsurface drip irrigation. However, a history of intense irrigation has also drained the Ogallala Aquifer and the recharge rate of water returning to the aquifer is at a deficit compared to the water being removed (Scanlon et al. 2012). Farmers in this region are now forced to reduce water inputs and find alternate crops that require less water. Cotton is grown primarily in the southern half of the High Plains because it requires less water than other crops like corn or soybeans (New & Dusek 2005). This already semi-arid region also has a history of severe bouts of drought including the notorious “dust bowl”; however drought conditions are expected to become more severe in the southern High Plains region due to the anticipated effects of climate change (Karl et al. 2009). At this point, it is unclear what impact these environmental fluctuations and lower water input will have on not only cotton crop health but also the residing arthropod communities in this economically important region.

Two of the earliest arthropods to colonize seedling cotton are the twospotted spider mites (*Tetranychus urticae*) and the western flower thrips (*Frankliniella occidentalis*). Both feed on cotton throughout the season, but controlling them early is critical, because feeding damage that is done during young plant development can result in inefficient plant growth due to branching (Sadras and Wilson 1998). These arthropod species are often found together on the same plant causing a high risk of damage during both the seedling and mature vegetative stages of cotton

(*Gossypium hirsutum*) (Wilson et al. 1983, Wilson et al. 1996, Pickett et al. 1988). Some behaviors of mites and thrips on a shared host plant have been documented, such as increased attractiveness of mite damaged plants to thrips colonization (Martini et al. 2013 and 2015), thrips using mite webbing as refuge from other predators (Pallini et al. 1998), and increased consumption of mite eggs by thrips through opportunistic feeding (Agrawal et al. 1999). However, no studies have examined the response of mixed communities of mites and thrips under drought stress. Population-level changes during drought could result from arthropod interactions, plant-arthropod interactions, or both. We expect drought to play a role in the overall performance of cotton seedlings through these interactions as well.

There is a high-level of complexity in the interactions among cotton and these two resident arthropod populations. With limited water resources as a present and future consideration in agriculture, their responses to drought conditions need to be explored to improve predictions of pest population dynamics for management strategies that go beyond direct effects of water stress on cotton, spider mites, and western flower thrips. Therefore, the objectives were to 1) evaluate the direct and indirect effects of drought on cotton seedlings and its pests and 2) determine if drought stress on plants is compounded or offset by changes in pest pressure resulting from indirect effects of drought on arthropod population dynamics. For Objective 1, we predicted that drought would directly reduce cotton seedling growth and development, as well as physiological changes in stomatal conductance, because of the need to retain moisture during dry conditions. Additionally, we predicted that drought stress in cotton would cause increased growth of mite populations because of enhanced developmental and reproductive enhancement, but lower thrips populations because of negative effects of the environment on thrips survival. For Objective 2, we predicted that arthropods would have a slight additional negative impact on

cotton seedlings during drought conditions because of thrips would not be able to significantly control mite populations; thus resulting in a predicted net increase in arthropod populations due to elevated leaf temperatures.

Materials and Methods

To achieve these objectives, we measured the population growth of western flower thrips and twospotted spider mites, as well as the growth, development and physiological activity of drought-stressed and well-watered seedlings of cotton with and without arthropods present in controlled greenhouse trials. Our greenhouse experiment was carried out with as a 2x2x2 factorial design: two water regimes (drought stressed, well-watered), two levels of twospotted spider mites (present or absent) and two levels of western flower thrips (present or absent). The split-split plot design contained four balanced replications, each of which had one experimental unit for each treatment combination. To increase the number of total replications for the study, the experiment was repeated at three different times with four replications each; the greenhouse space was not large enough to run all replications at one. Repeating the experiment provided a total of 12 replicates of each treatment combination.

The amount and frequency of irrigation were adjusted to achieve drought-stressed and well-watered cotton seedlings at the development of the first-true leaf. The low water or "drought" treatment simulated drought conditions with low water inputs from irrigation. The well-watered treatment simulated non-limited irrigation or normal "well-watered" conditions. Plants from both water treatments were exposed to western flower thrips and/or twospotted spider mites, including a no-arthropod control.

During the experiment, both destructive and non-destructive samples were taken. Arthropod data events are represented in this thesis as *Sample 1* (taken 11 days after arthropods

were inoculated) and *Sample 2* (taken 18 days after arthropod inoculation). Plant data events are represented in this thesis as *pre-arthro reading (taken before the inoculation of arthropods)*, *post-arthro reading 1* (taken 11 days after arthropods were inoculated), and *post-arthro 2* (taken 18 days after arthropod inoculation). The destructive samples provided exact counts of arthropods as well as plant measurements from each set of plants, while the non-destructive samples provided continuous plant data. For each treatment combination, plant and arthropod responses were measured as described below.

Cotton plant enclosure system

The cage organization was evenly spaced and oriented east-west. This orientation was selected to produce even airflow among the plants in the greenhouse. Metal halide lights were spaced evenly to produce a uniform 14:10 light-dark photoperiod throughout the greenhouse space. Additionally, OnSet® HOBO devices (Onset Computer Corp., Bourne, MA) gathered temperature and relative humidity data during the experiment. The average 24-hour temperature in Celsius was 24.16 ± 0.03, 25.19 ± 0.04, and 23.49 ± 0.03 for trials one, two, and three, respectively. The average relative humidity in percent was 30.36 ± 0.08, 33.59 ± 0.13, and 24.29 ± 0.02 for trials one, two, and three, respectively.

Each cage used in the experiment was composed of two components: (1) PVC soil column and (2) exclusion cage (Figure 1). First, the vertically standing PVC column (60 cm tall by 15 cm in diameter) had wire mesh attached to the bottom opening of the PVC column for soil support. Next, a coffee filter was placed inside the column on top of the wire mesh followed by 1,200 grams of air-dried sand. Then, columns were slowly loaded with 11,400 grams of field soil, which afforded adequate headspace for irrigation. The preceding wire mesh, filter, and sand thwart the loss of the growing substrate. Second, an exclusion cage (81 cm tall by 35 cm wide at

the top and 23 cm wide at the base), bound by polyester mesh (120 micron- 34% open area, mesh No.07-120/34 from ELKO Filtering Company, Tamarac, Florida), was later attached to the top of the PVC column immediately after seeds were watered. This cage had a 76 cm zipper sewn vertically into the mesh for ease of access and a clear plastic ceiling for light penetration. This cage design provided adequate space for the cotton to grow both above and below the soil surface for the length of the experiment.

Field soil was used in the experiment as a growing media to provide the water holding potential, chemical makeup, and microbial community of the natural conditions cotton would experience in the agricultural region of interest. The field soil was collected from a cotton field in Moscow, Kansas (GPS coordinates: 37.314996, -101.093177) and is described by the USDA as a Zella loam (https://websoilsurvey.sc.egov.usda.gov/). All soil was put through a soil grinder to ensure uniform particle size.

Seedlings used in the experiment were grown, one-per-cage, and irrigation amounts were determined by weighing the column. A single OHaus® weighing scale (RangerTM 3000 series, Davidson, North Carolina) was randomly assigned to one cage within each four-cage area. The scales allowed us to determine the amount of water to add to these four cages. At the start, each soil column received 570 grams of water added to the soil surface regardless of its assigned treatment. Because the water is added to the soil surface and is absorbed by the top-most portion of the soil matrix, this water quantity equated to 15% water content by weight in the top 18 cm of soil, which was the approximate root zone for cotton seedlings. Next, five cottonseeds were planted and then thinned back to one healthy seedling, which was to be the experimental plant. The silty-clay texture of the field soil often hardened into an impenetrable crust, which caused non-uniform or irregular epigeal germination (emergence above the soil surface). To avoid this

issue, we carved shallow, parallel grooves into the soil surface before planting. Until seeds germinated and developed expanded cotyledons, the weight of water lost from the column was added back to the soil surface daily to maintain proper germination conditions. At this preparatory stage, drought-stress had not yet been initiated.

Achieving drought stress before arthropod inoculation

Once the cotyledons expanded and the first true-leaf was visible, the timing of irrigation events was determined by the weight of columns throughout the remainder of the experiment. When the soil columns of plants designated as well-watered reached 70% of their original water weight at the beginning of the experiment, we added enough water to reach 100% original water amount. In contrast, when soil columns of plants designated as drought stressed reached 50% of their original water weight, they were given only enough water to reach 70% of the original water amount. This resulted in a clear difference in water availability for each of our water treatments (Figure 2). By using these methods, we properly simulated in a controlled environment the water holding potential and characteristics of field conditions experienced in the High Plains.

Arthropod colony development

Arthropods were reared in colonies before being used in the experiment. Mites that were used in the study originated in field corn (*Zea mays*) from Garden City, Kansas, and reared on seedling corn. Over the course of the next year, mites were selected for survival on cotton seedlings (Bayer '121353', insecticide-free seed) by adding mites to the colony on a weekly basis. In a separate growth chamber at 27°C with a 16-hr light:8-hr dark light regime, cotton seedlings were grown without mites to the fourth true-leaf stage and watered as needed to keep

them well hydrated. Then they were transferred to the completely enclosed mite colony cages in the greenhouse. Fresh plants were regularly exchanged for heavily damaged and older cotton plants because they not optimal hosts for twospotted spider mites (Karban and Thaler 1999).

Thrips used in the experiment originated in soybeans (*Glycine max*) from Garden City, Kansas. They were raised in the laboratory on green beans (*Phaseolus vulgaris*) in 15 cm by 15 cm plastic containers. A 7-cm hole in the lid of the container was fixed with polyester mesh (120 micron- open area %:34, mesh No.07-120/34; ELKO Filtering Company, Tamarac, Florida) to allow airflow, enclose thrips, and exclude unwanted arthropods. Artificial metal halide lighting was set to a 16-hr light:8-hr dark photoperiod, and room temperatures averaged approximately 24°C.

Data collection and arthropod inoculation

When every plant had a fully expanded first true-leaf, plants assigned the *mites only* or *mites and thrips* treatments received three newly emerged adult female mites via a small cotton plant leaf-disc. *Thrips only* and *mites and thrips* treatments receive a total of 50 thrips from two rounds of inoculations of 25 first-instar thrips two and four days after mite infestation to replicate natural, early-season infestation patterns in cotton fields. First-instar thrips were used because of their higher predation rates on twospotted spider mite eggs than that by adult thrips (Trichilo and Leigh 1986). They were randomly selected from the thrips colony and, therefore, not sexed. The delay between inoculations of mites and thrips provided the egg life-stage of mites for consumption by thrips. Thrips, especially first-instar thrips, have not been documented feeding on non-egg life stages of the mites.

Arthropod population numbers and thrips scars, the damaged area of a plant to thrips feeding, were recorded on a per plant basis: mite eggs, mobile mites (i.e., all life stages except

egg), total mites (i.e., all life stages), total thrips (i.e., all life stages except eggs), and total number of scars caused by thrips. Counting arthropods required sampling plants destructively; hence the utilization of two separate samples one week apart (page 9). During sampling events, leaves were cut from the experimental plants and stored at -80 °C until later processing took place. To estimate a leaf's total mite populations, life stages were counted on half of each leaf and multiplied by two. These leaf estimates were then summed to get a total quantity per plant. This estimation technique was necessary because of the large mite population sizes and time restrictions. Thrips populations and thrips scars were not estimated because their populations were manageable. Discrete counts of thrips and thrips scars were recorded immediately after the plant was sampled. Yellow sticky cards (11 cm x 13 cm) were placed inside each exclusion cage for 7 days to catch mites or thrips that may have remained in the cage after sampling. These captured numbers were added to their respective data category.

Stomatal resistance (seconds per meter), leaf surface temperature (Celsius), and leaf surface relative humidity (%) were recorded using non-destructive readings on the abaxial leaf surface using a Decagon® steady state porometer (Model SC-1, Decagon Devices, Pullman, WA). Readings were taken on the youngest, fully expanded true-leaf and between 12 pm and 1 pm to avoid the effects that shade and photoperiod can have on porometer readings (Monteith et al. 1965). These readings were taken on every plant at three different times during the experiment: immediately before the inoculation of mites (*pre-arthro reading)*, 11 days after inoculation (*post-arthro 1 reading)*, and 18 days after inoculation (*post-arthro 2 reading)*. Plant height and the number of leaf nodes were also recorded at these events. Note that the post-arthropod readings were performed on the same days as the arthropod samples. All plant data were recorded before destructive sampling for arthropod data. Lastly, primary root length was

record at the termination of the experiment by measuring the length from the root crown to the end of the primary root. Extraction of the root required gentle, manual removal of soil into a pan and, when done while the plant is still fresh and green, the root was easily pulled from the soil profile.

Statistical analysis

An analysis was performed to test the consistency of the greenhouse environment during the three greenhouse trials to justify pooling the data from the twelve replicates of the study. Average day conditions varied between the three trials by 0.91°C and 6.47% relative humidity. Average night conditions varied between the three trials by 0.74°C and 10.88% relative humidity. We considered these differences between the environmental conditions for which we ran our three trials negligible to the performance of our experiment, justifying the pooling of our data across the twelve replicates.

The consistency in experimental design, environment, and methods performed during the three trials of this study allowed us to confidently determine that the statistical variations between the three trials were normal. Additionally, these statistical differences across trials did not follow any particular trend that would signal a reason to abstain from pooling the data. In other words, these differences were random and did not follow a trend that correlated to any particular arthropod, plant, or climate factor.

To determine how drought, spider mites, and thrips influenced plant development and physiology, plant data from the twelve replications were pooled. Within this pooled data, the three plant reading dates (*pre-arthropod reading, post arthropod reading 1,* and *post arthropod reading 2*) were analyzed as separate events using PROC MIXED (allows the data to exhibit correlation and nonconstant variability) as a generalized linear model with Kenward-Roger

degrees of freedom approximation in SAS 9.4. All combinations of water and arthropod treatments were compared for least square mean differences; the pre-arthropod inoculation analysis excluded the arthropod treatments and only functioned as a test for differences between water treatments.

A separate analysis was performed on the plant trait data using a repeated measurement approach where a sub-set of 32 plants was measured during each of the two reading dates to measure the plants' traits over the duration of the experiment. PROC MIXED (allows the data to exhibit correlation and nonconstant variability) with Kenward-Roger degrees of freedom approximation in SAS 9.4 was used to compare water and arthropod treatments at each of the reading dates. This repeated measures analysis yielded the same results of main effects on plant traits as the pooled plant analysis.

Spider mite, thrips populations, and thrips scar counts from the twelve destructively sampled replications were each pooled separately by sample date and analyzed using PROC GLIMMIX (allows the data to exhibit correlation and nonconstant variability) as a generalized linear model with a negative binomial distribution and a log-link function in SAS 9.4. Combinations of water, arthropod, and sample date were compared for least square mean differences. Mite life stages were analyzed by the three categories of mite eggs, mobile mite quantity, and total mite quantity. Thrips were analyzed by one category of total thrips quantity. Alpha significance level was 0.05 for all analyses.

Results

Effect of drought and arthropods on plant development and leaf status

Plant height was significantly affected by water treatment at each of the three readings (*pre-arthropod*, *post-arthro 1*, and *post-arthro 2*), but was unaffected by arthropods or the combination of water and arthropods at any point during the study (Table 2.1). Also, sample date significantly affected plant height, which means the plants grew taller as the experiment progressed (Table 2.1). Drought stressed plants were 31 cm, 90 cm, and 127 cm shorter than well-watered plants at the *pre-arthro*, *post-arthro 1*, and *post-arthro 2* readings, respectively (Figure 2.3). However, there was no significant difference in plant height from the arthropod treatment at either of the arthropod sampling dates (Table 2.1).

The number of leaf nodes was significantly affected by water treatment at each of the three readings. Specifically, drought-stressed plants had progressively fewer nodes than well-watered plants at each sampling date (Figure 2.4). Arthropod treatment at *post-arthro 2* reading also significantly affected leaf nodes, with calculated a difference between the highest and lowest means of 0.35 nodes (Figure 2.5). However, there was no significant interaction of the main effects of water and arthropod (Table 2.1). Sample date significantly affected leaf nodes during the experiment for both well-watered and drought-stressed plants, with more nodes on the second sample date (Table 2.1).

Stomatal resistance was significantly affected by water treatment at each of the three readings. Drought stressed plants had high stomatal resistance readings, which ranged from 853-1292 s/m across the three samples, while well-watered plants had low levels of stomatal resistance, ranging from 220-409 s/m (Figure 2.6). Stomatal resistance was significantly greater with arthropods present than when they were absent, but only at the *post-arthro 2* reading. The

mean stomatal resistance of the no arthropod control plants was 604 s/m while those of the arthropod treatments were >900 s/m (Figure 2.7). There was no significant interaction between water and arthropod treatments with respect to stomatal resistance on either sampling date (Table 2.1). Sampling date also significantly affected stomatal resistance (Table 2.1). Because there was a significant arthropod effect on stomatal resistance, we wanted to determine if stomatal resistance became greater as arthropod populations increased. A correlation analysis showed no correlation between mite populations and stomatal resistance ($r = -0.229$, $N = 12$, $P = 0.474$, $t = -0.745$, $df = 10$), and no correlation between total arthropod populations (mites + thrips) and stomatal resistance ($r = 0.189$, $N = 12$, $P = 0.556$, $t = 0.609$, $df = 10$).

Leaf relative humidity was significantly affected by water treatment at each of the three readings. Leaf relative humidity of drought plants was lower (range: 60-64 %) compared to well-watered plants (range: 73-75 %) (Figure 2.8). Leaf relative humidity was also affected by arthropod treatment, but only at the *post-arthro 2* reading. It was slightly, but significantly, lower in the arthropod treatment (mean = 70 %) compared to the no-arthropod control (mean = 67 %) (Figure 2.9). There was no significant effect of water*arthropod treatment at any point during the experiment (Table 2.1). Sampling date did not significantly affected leaf relative humidity.

Leaf temperature was significantly affected by arthropod treatment on both sampling dates (Table 2.1). Minimum and maximum leaf surface temperatures differed by 0.26 ˚C at *post-arthro 1* and by 0.42 ˚C at *post-arthro 2* readings in treatments with and without arthropods present (Figure 2.10); the leaf temperature was lowest when arthropods were absent. For each sample date, leaf temperature was more variable when arthropods were present. There were no significant differences in leaf temperature due to water treatment or the combination of water *

arthropod treatments on either sample date (Table 2.1). Sampling date also had no effect on leaf temperature (Table 2.1).

Root length was not significantly affected by the water, arthropod, or the combination of these treatments at any point during the experiment (Table 2.1).

Effect of drought on arthropod populations

Spider mites. There was no difference in mite population response to the water treatments 11 days after inoculation (Sample 1). However, while there were significant increases in mite populations from Sample 1 to Sample 2 on both well-watered and drought-stressed plants, drought treatment plants showed reduced increase of both mite egg and mobile mite populations compared to the well-watered treatment. Drought plants experienced an eightfold increase in mite eggs and mobile mite numbers doubled, whereas well-watered plants experienced an approximate 19-fold increase in mite eggs and mobile mites quadrupled (Figure 2.11 and 2.12, respectively). When thrips were also present, mite populations on drought-stressed plants, but not well-watered plants, had significantly fewer eggs and mobile mites 18 days after inoculation of mites compared with similarly treated plants without thrips (Figures 2.11 and 2.12).

Thrips. Thrips populations were not affected by water conditions at any point during the experiment (Table 2.1). However, thrips populations were negatively affected by the presence of mites at Sample 2, with a thrips population reduction of 37% (Figure 2.13). The numbers of thrips scars, or damaged areas from feeding, present on the cotton plant leaves were not affected by water or arthropod treatments on either sampling date (Table 2.1).

Discussion

Drought stress had direct and indirect effects on both the plant and the arthropods. Cotton plants responded directly to drought as expected by slowing growth and development, and physiological regulation of stomata (closure under drought conditions). Although cotton seedlings are adapted to tolerate drought by conserving water and energy by these responses, doing so for long periods may have adverse effects on plant performance if drought conditions persist further into the growing season. For example, reduced plant height and fewer leaves result in less shade produced by each plant. This would allow heat to accumulate beneath the canopy and between plants within the crop rows, furthering water loss in the plant. Although we did not observe a direct increase in temperature at the leaf surface due to drought, it was slightly higher than the temperature measured in the greenhouse space, meaning that the leaves were retaining heat (Jackson 1982). This is a result of stomatal closure (Hetherington and Woodward 2003), which was observed as a direct response to the drought treatment. Determining if a plant is experiencing drought stress using only leaf surface temperature may not capture the suite of physiological responses. If we had continued our study into later stages of plant maturity, then we may have observed distinctly different leaf temperatures between water stressed and non-stressed plants.

Evidence of indirect effects of drought on plant performance were recorded as differences in plant responses caused by the arthropod treatments. Arthropods had a negative impact on cotton seedlings in both water treatments for all plant traits except plant height. However, the effects were greatest in the drought treatments. From this we infer that arthropod feeding compounded drought stress. With respect to plant growth, the no-arthropod control had significantly more leaf nodes in both water treatments than the plants that had mites, thrips, or

both (Figure 2.5). Although this difference may not be detrimental to the survival of the seedling cotton plant, it would likely affect timing of cotton boll and fiber development as the plant matures (Sadras and Wilson 1998). Postponed crop harvest due to delayed crop development creates financial risks for the producer.

Arthropods affected the physiological function of the plant – specifically, stomatal resistance, leaf temperature, and leaf relative humidity. This is in line with what we predicted, because these three plant responses are interrelated (i.e., leaf temperature and humidity are influenced by stomatal function). All of these effects signal that there were changes in stomatal function resulting from arthropod infestation. We recorded lower stomatal resistance with arthropods present compared to the no-arthropod control early in the experiment (*post-arthro 1* reading), but arthropods were associated with higher stomatal resistance compared with the no arthropod control later in the experiment (*post-arthro 2* reading) when mite and thrips populations were much higher (Figure 2.7). In general, we would expect to find lower stomatal resistance early in the experiment when drought and arthropod stresses were lower because the stomatal pores of the leaves can be open to allow for water exchange and function properly. However, it is unclear why stomatal resistance was lower on the first sampling date with arthropods present compared to uninfested plants. Leaf temperature and leaf relative humidity readings fluctuated in their response to the arthropod treatments from one reading to the next during the experiment. We suggest that the stomata were damaged by arthropod feeding, which would change the plants' ability to regulate gas exchange and leaf temperature (Holtzer et al. 1988). Over 18 days we were limited to three readings to avoid disturbing the arthropod populations, which provided a brief glimpse into the continuous fluctuations that would have

occurred. Future studies would benefit from the development of methods for continuous leaf measurements to provide more thorough insight into the influence of arthropods on leaf function.

Mite populations appeared to experience reduced rates of growth from both drought stress and the presence of thrips. These population shifts were not observed until *Sample 2*. To help explain why the populations diverged as the experiment progressed, bottom-up influence (from the plant on mite populations) must be discussed. Increases in leaf temperature and relative humidity probably played an insignificant role in the differences in mite populations because the differences in these measurements between the two water conditions fell within a narrow range that would not result in alterations to arthropod populations (Margolies & Wrensch 1996, Steenbergen et al. 2018). Thus, these environmental conditions are unlikely to have been the major drivers for distinct mite population sizes between our water treatments.

We believe a potential indirect effect of drought that may have influenced mite populations is a decrease in host plant quality as a food source. Because of the reductions in mite populations in the drought treatment with *mites only*, we can rule out any predatory effects of thrips. We suggest host plant quality as a possible explanation because cotton seedlings experienced drought stress throughout the study that resulted in changes in plant development and physiological performance. Jasmonic acid is a plant defense hormone that is expressed when a plant is damaged by herbivorous arthropods including spider mites. This compound has been shown to reduce mite feeding and mite egg production on host plants when expressed *in vivo* or applied as a foliar spray (Li et al. 2002, Choh et al. 2004). Two additional compounds linked to jasmonic acid are abscisic acid, which is released under drought stress to regulate stomatal aperture, as well as feeding damage caused by thrips to resist their feeding (Thaler 1999, Abe et al. 2008). Furthermore, although drought stressed plants can have elevated levels of nitrogen,

which is beneficial to the diet of mites, access to these nutrient compounds by mites' piecing-sucking mouthparts for feeding were likely impeded by the reduced turgor pressure of the leaf (Huberty & Denno 2004). Having multiple factors of drought and arthropod feeding contributing to plant defenses, and, therefore, a poorer food source, could explain why mite populations were not as prolific as those on well-watered plants in our study.

The reduced growth of mite populations was compounded by the presence of thrips in drought conditions, but not in well-watered conditions (Figure 2.11 and 2.12). We believe that another potential indirect effect of drought on mite population growth in our study is predatory pressure from thrips and/or competition between thrips and mites for resources. For example, consumption of spider mite eggs by western flower thrips has been documented in previous studies (Janssen et al. 2013, Agrawal et al. 1999), and Chapter 3 of this thesis recorded thrips consuming more mite eggs under drought conditions while continuing to feed on the same amount of leaf tissue. Direct interference competition is a potential cause of these reductions.

Thrips populations were negatively affected when they shared a host plant with mites rather than when they were alone, but were unaffected by water treatment (Figure 2.13). These results suggest that any influence of drought on thrips was a result of competitive interactions with mites and not due to changes in host plant quality for thrips or physical environmental conditions. Because we found that mite and thrips populations were smaller in the presence of each other under both well-watered and drought settings (Figure 2.11 and 2.12), we believe that competition for resources such as space and food likely played the most significant role in reduced arthropod populations. Both spider mites and thrips prefer clean plant regions that are not shared with neighboring arthropods or predators (Pallini et al. 1997, Pallini et al. 1998). Each arthropod may have avoided regions with the other, which would have caused issues for them

from the limited amount of plant tissue of the small cotton seedlings. Reductions in both populations suggest that the shared space of the host plant was a limiting factor for the two arthropod populations. Future studies that examine further into the growing season of cotton will be able to determine how thrips populations would develop over time with more space and changing plant-arthropod dynamics.

Conclusion

We conclude that some of our predictions were accurate and others were not because of the complexity of responses of the cotton seedlings and the arthropods under the drought conditions we imposed.

We accurately predicted that drought would directly influence plant development and physiological functions. Seedling cotton was negatively impacted by drought through stunted growth and development, which would delay and reduce fruiting as the plant matures. We also predicted that drought would have indirect effects on plant stress due to changes in arthropod populations. This prediction was accurate in that we recorded reductions in mite populations (but not thrips) due to drought, and that drought indirectly affected plant nodes, stomatal resistance, leaf temperature, and leaf relative humidity through arthropod responses (Table 2.1).

When evaluating Objective 2's question of whether the effects of drought are compounded or offset by changes in pest populations, it is important to look at the net effect of drought on the plant. We see negative effects on most plant traits when pest populations were present, and negative effects from drought on plant development and physiology. During the early season, this suggests that the net effect of drought on seedling plants was *compounded* by arthropod responses. Seedling cotton is noted as being able to outgrow arthropod damage after it develops beyond the seedling stage (Leigh et al. 1996), but we suspect that this resilience may be

compromised under a persistent drought. Therefore, future studies should extend the duration of the observations on cotton, including fruit production and quality. For example, imposing drought at the level we tested did not make the plant more vulnerable to death compared to well-watered seedlings. In addition, our study limits our ability to provide details on important topics such as development time for crop maturation and yield rates.

Figures

Figure 2.1 Cage, including exclusion enclosure and PVC soil column, sitting on top of an Ohaus® weighing scale.

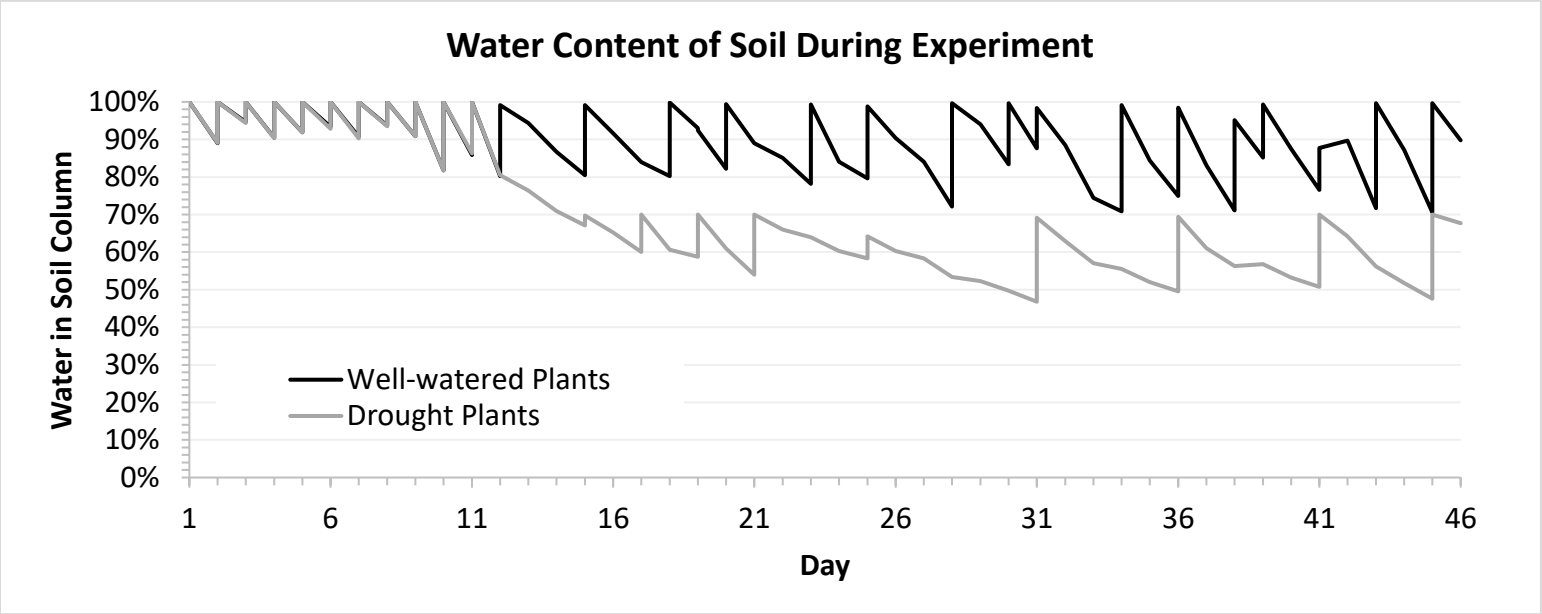

Figure 2.2 Daily fluctuation of soil moisture between the two water treatments during one round of the experiment. Percentage is calculated from original starting weight of 570 grams of water added to the soil. Vertical line segments indicate irrigation events. Lower-case letters *a-e* indicate prominent events: (*a*) cottonseed planting, (*b*) initiation of different water treatments, (*c*) Day 0 = pre-arthropod plant reading immediately followed by mite inoculation, (*d*) Day 11 = arthropod *Sample 1* and plant *post-arthro 1*, and (*e*) Day 18 = arthropod *Sample 2* and plant *post-arthro 2*, and, thus, the termination of the experiment.

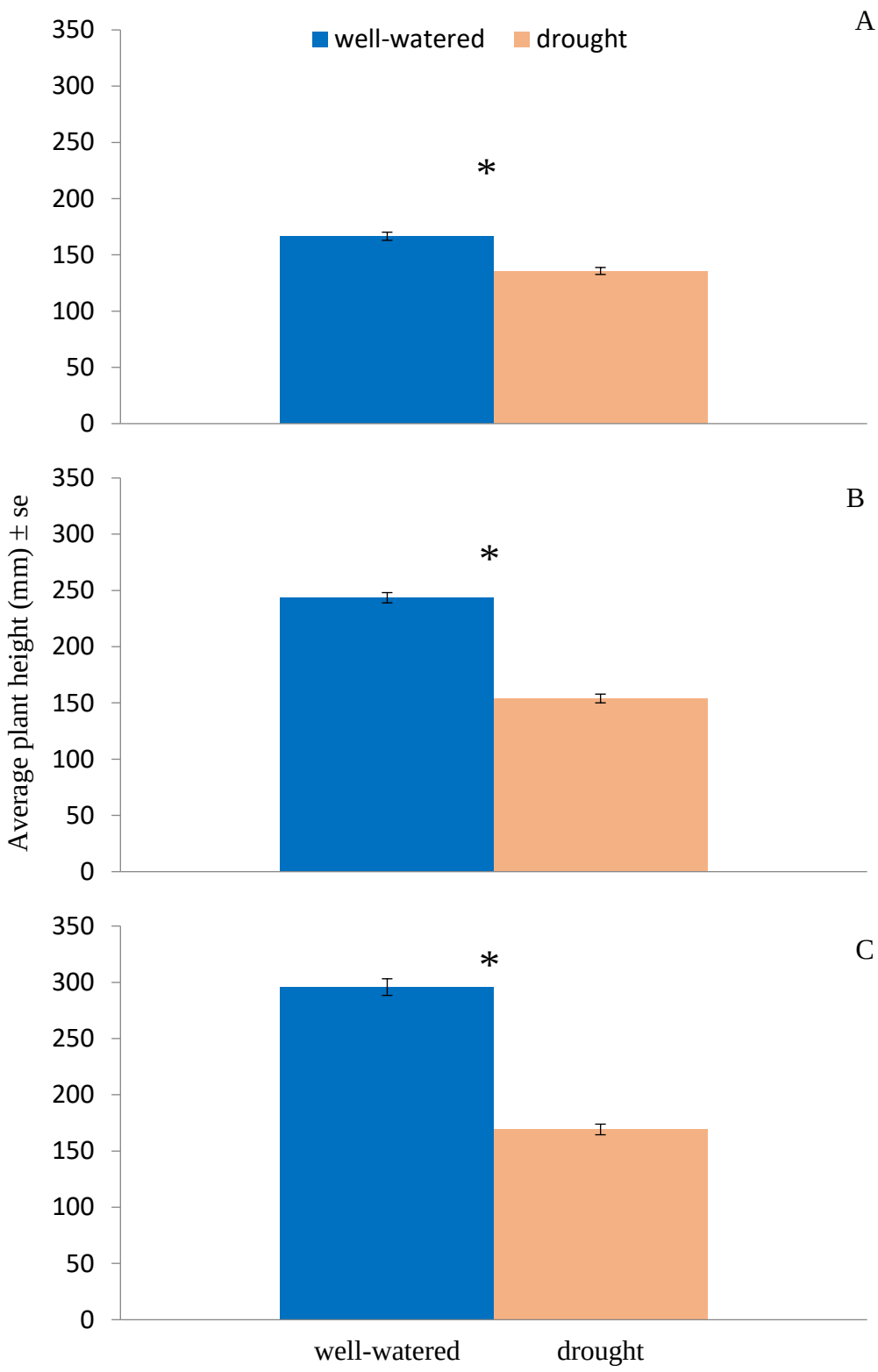

Figure 2.3. Plant height in millimeters of well-watered and drought stressed plants at three different readings: (A) *pre-arthropod* reading, (B) *post-arthro 1* reading, and (C) *post-arthro 2* reading. Asterisks indicate statistical significance ($p<0.05$).

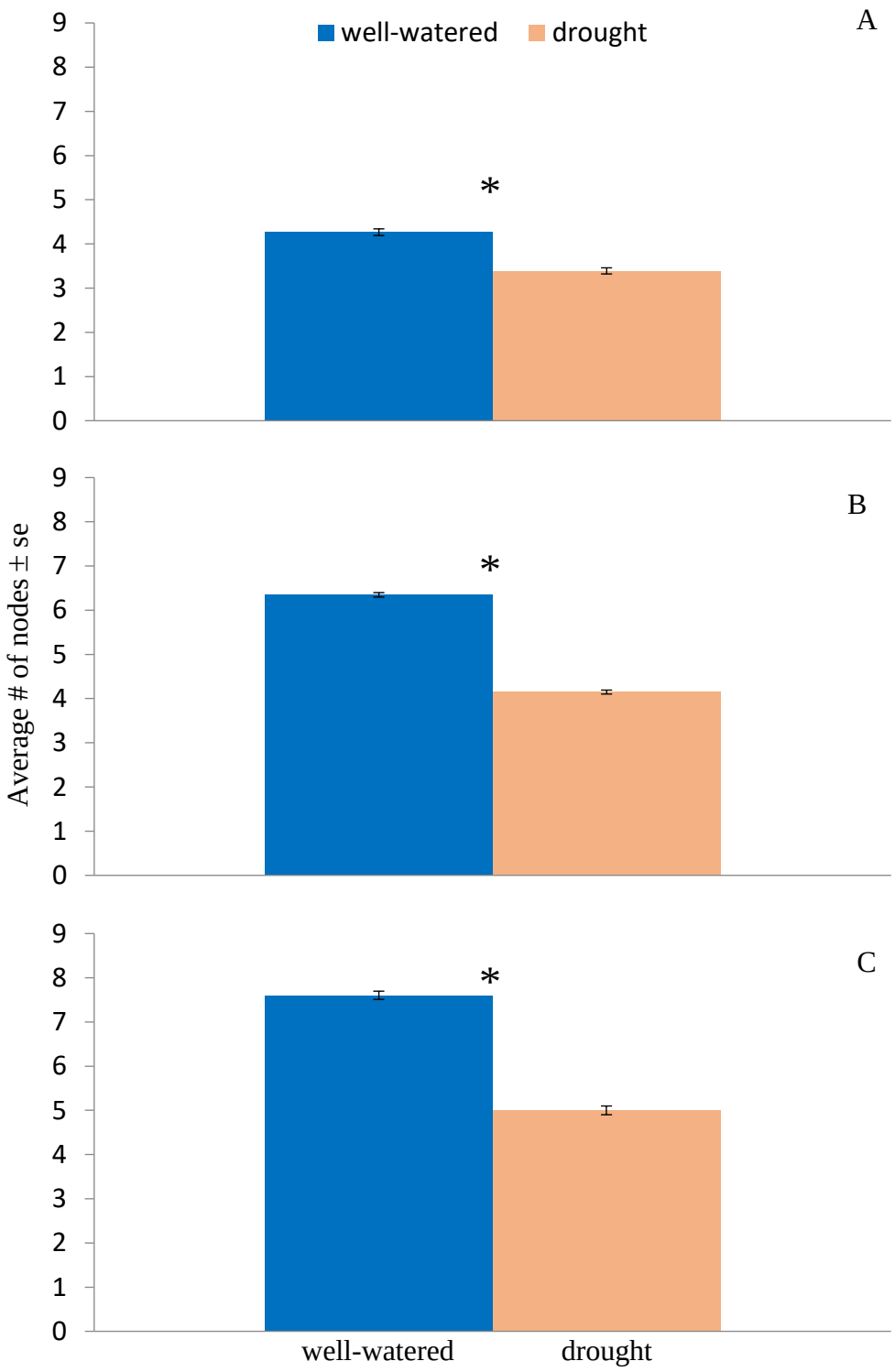

Figure 2.4 Leaf nodes of well-watered and drought stressed plants at three different readings: (A) *pre-arthropod* reading, (B) *post-arthro 1* reading, and (C) *post-arthro 2* reading. Asterisks indicate statistical significance ($p<0.05$).

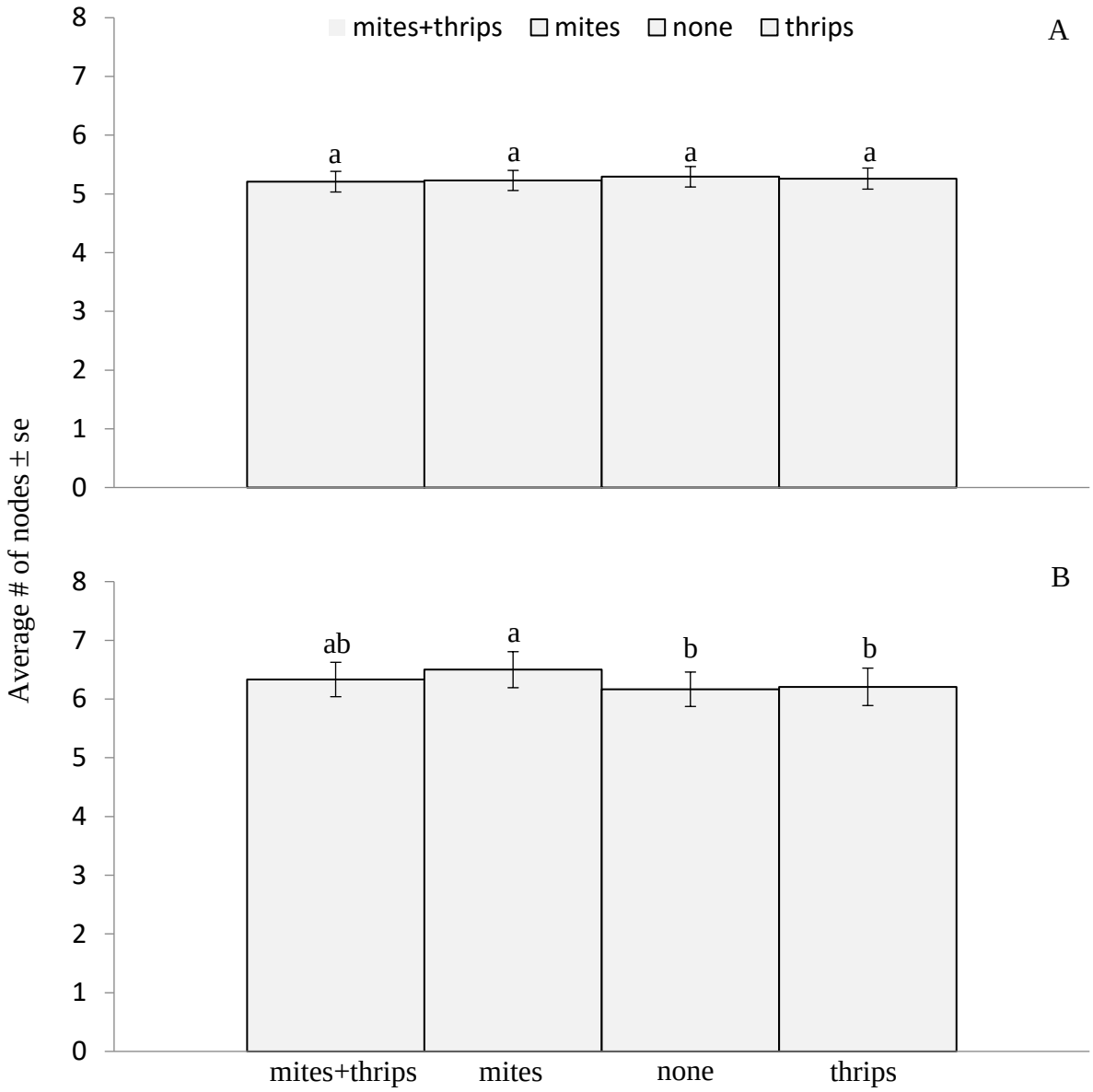

Figure 2.5 Number of leaf nodes of plants with each arthropod treatment at two different readings: (A) *post-arthro 1* reading and (B) *post-arthro 2* reading. Lowercase letters signify an across-arthropod treatment comparison. Significance set at $p<0.05$.

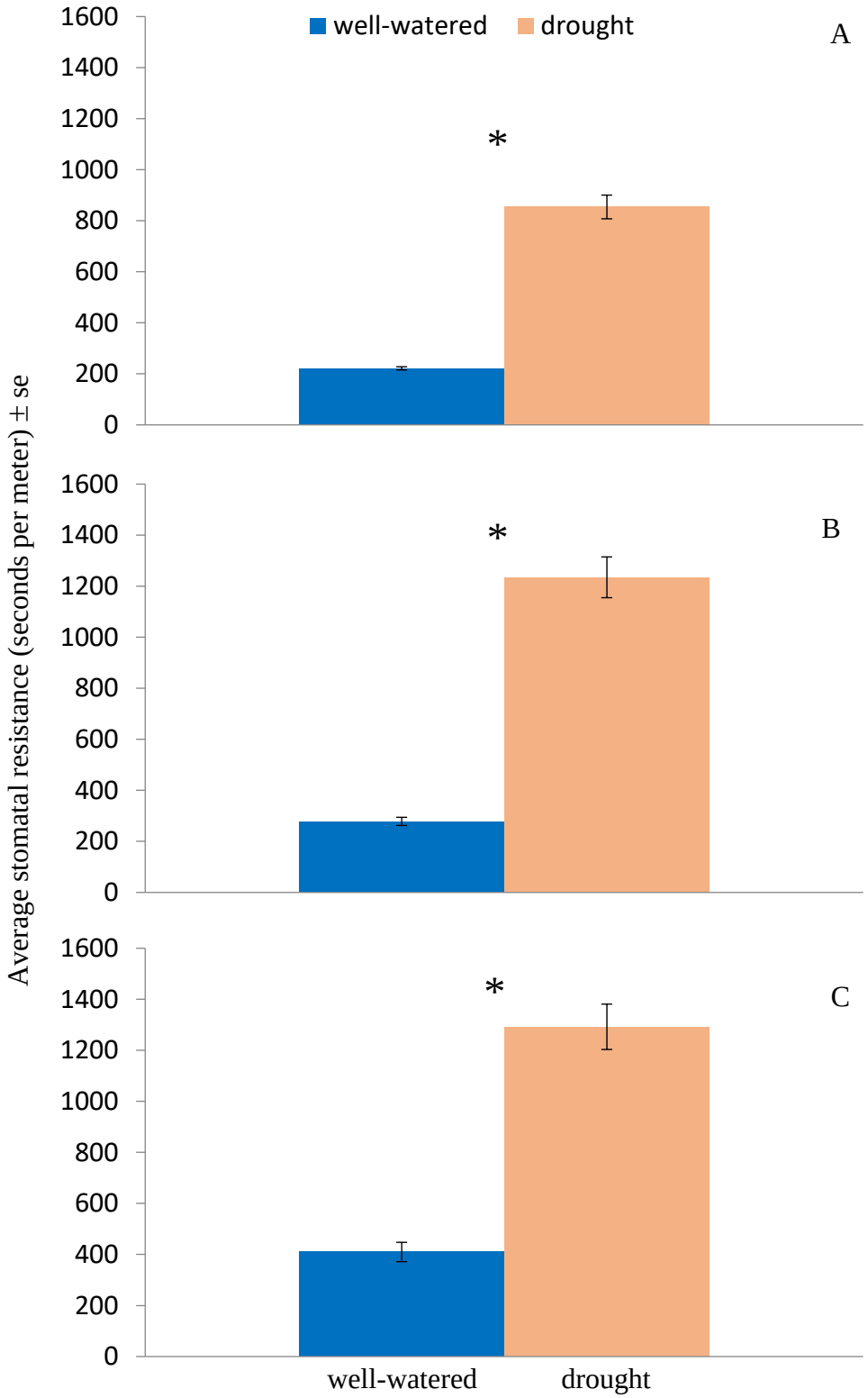

Figure 2.6 Stomatal resistance in seconds per meter of well-watered and drought stressed plants at three different readings: (A) *pre-arthro* reading, (B) *post-arthro 1* reading, and (C) *post-arthro 2* reading. Asterisk indicates statistical significance ($p<0.05$).

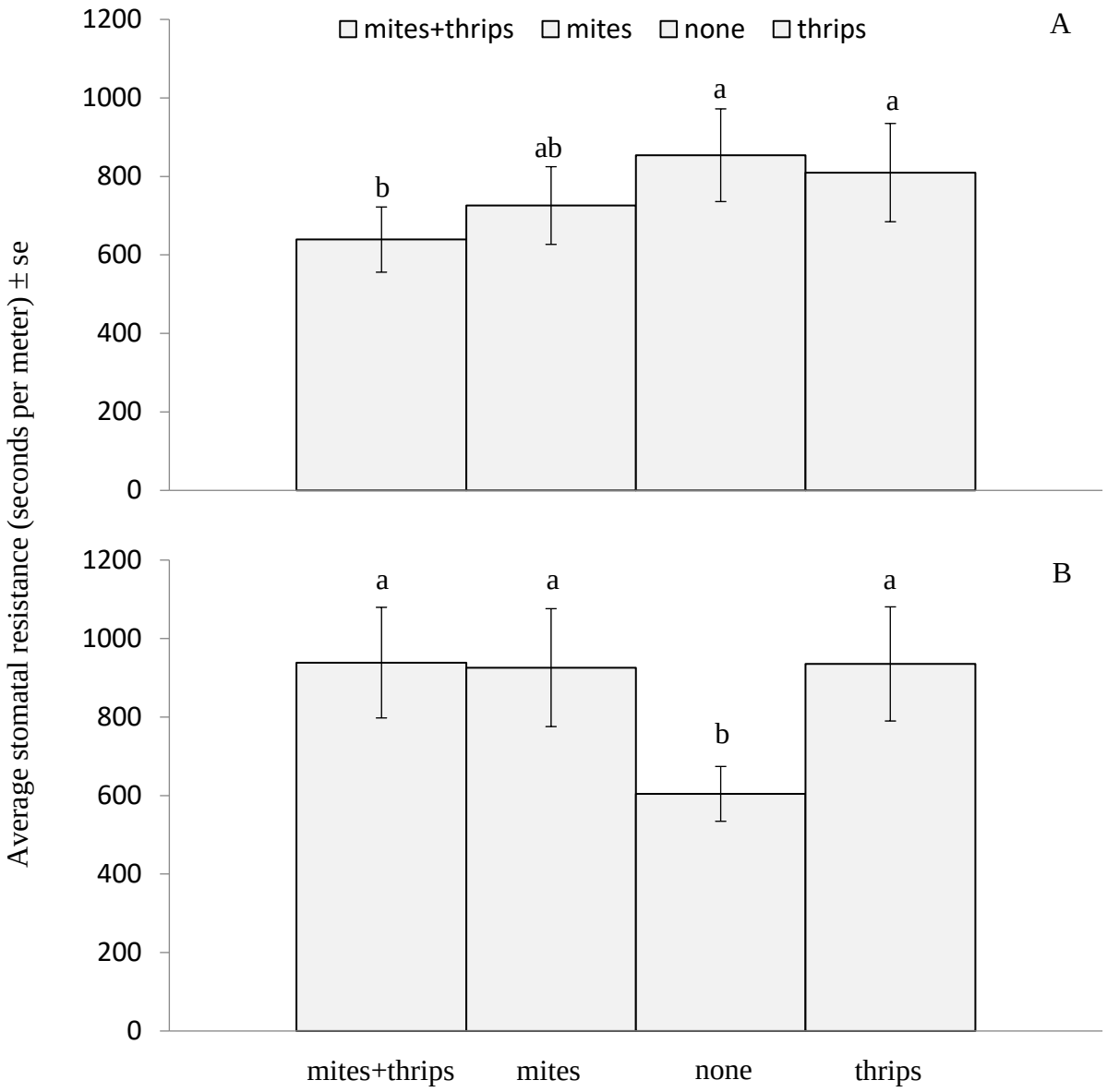

Figure 2.7 Stomatal resistance in seconds per meter of plants with each arthropod treatment at two different readings: (A) *post-arthro 1* reading and (B) *post-arthro 2* reading. Lowercase letters signify an across-arthropod treatment comparison. Significance set at $p<0.05$.

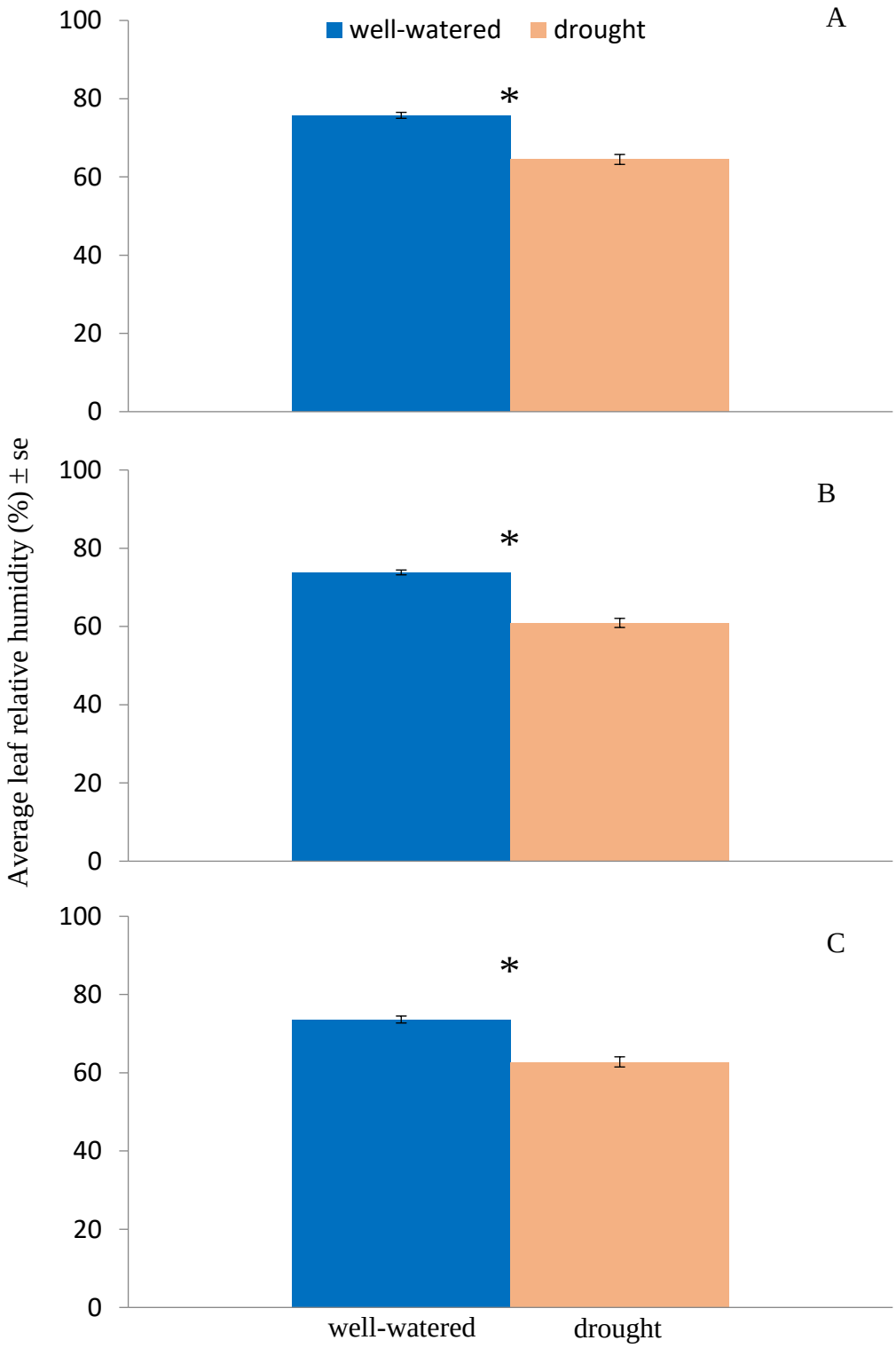

Figure 2.8 Leaf surface relative humidity in percent of well-watered and drought stressed plants at three different readings: (A) *pre-arthro* reading, (B) *post-arthro 1* reading, and (C) *post-arthro 2* reading. Asterisk indicates statistical significance ($p<0.05$).

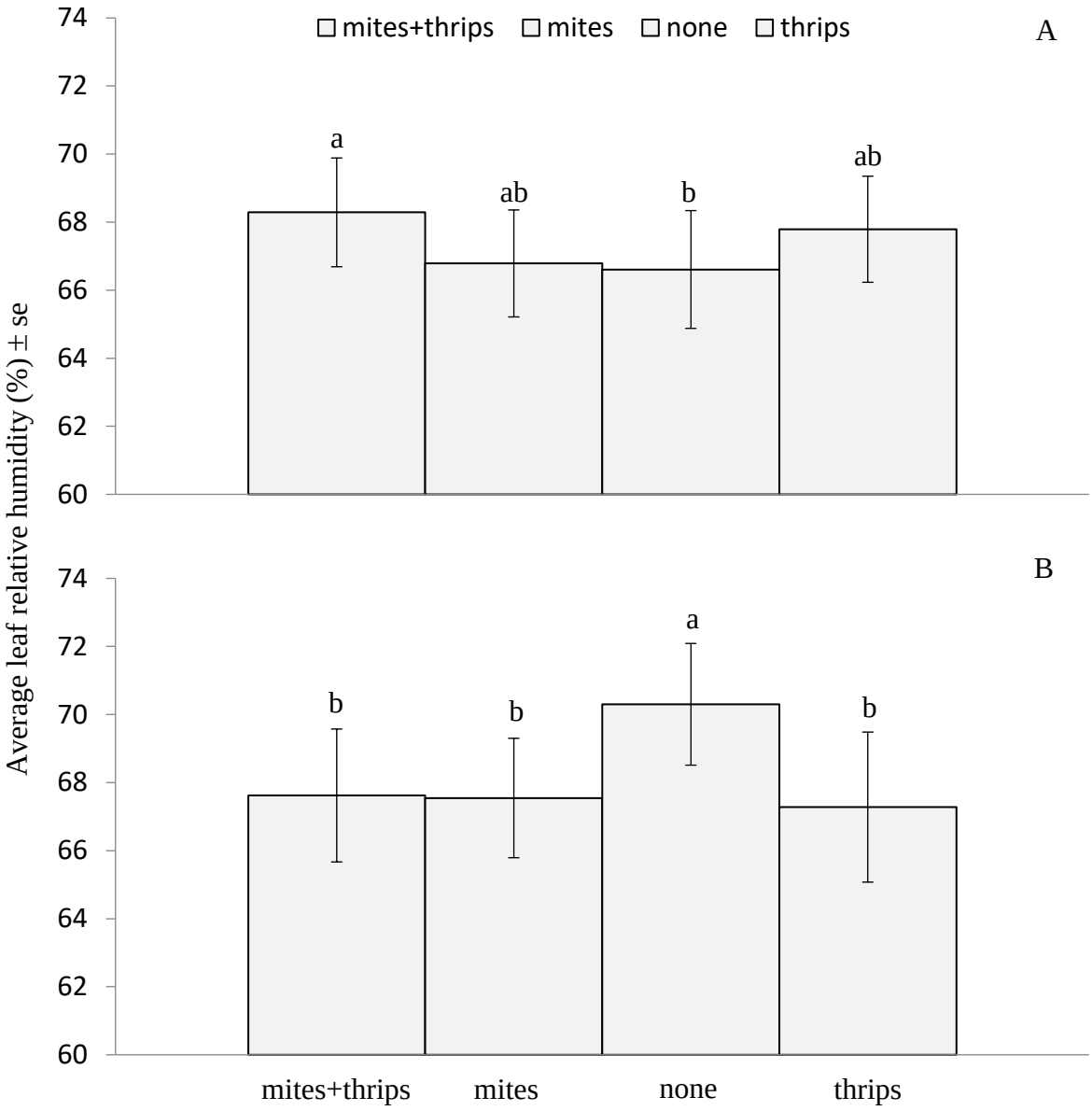

Figure 2.9 Leaf surface relative humidity in percent of plants with each arthropod treatment at two different readings: (A) *post-arthro 1* reading and (B) *post-arthro 2* reading. Lowercase letters signify an across-arthropod treatment comparison. Significance set at $p<0.05$.

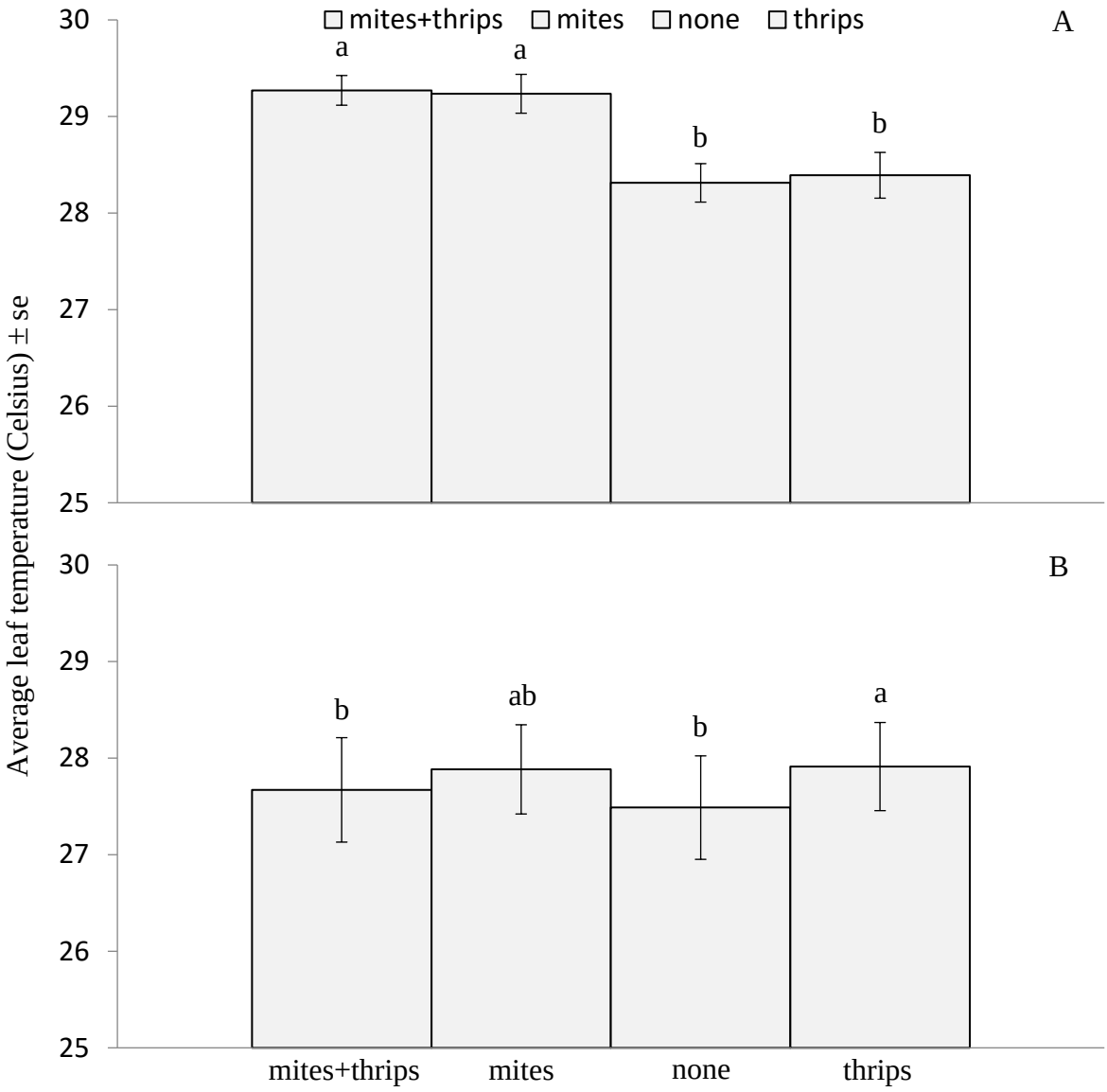

Figure 2.10 Leaf surface temperature in Celsius of plants with each arthropod treatment at two different readings: (A) *post-arthro 1* reading and (B) *post-arthro 2* reading. Lowercase letters signify an across-arthropod treatment comparison. Significance set at $p<0.05$.

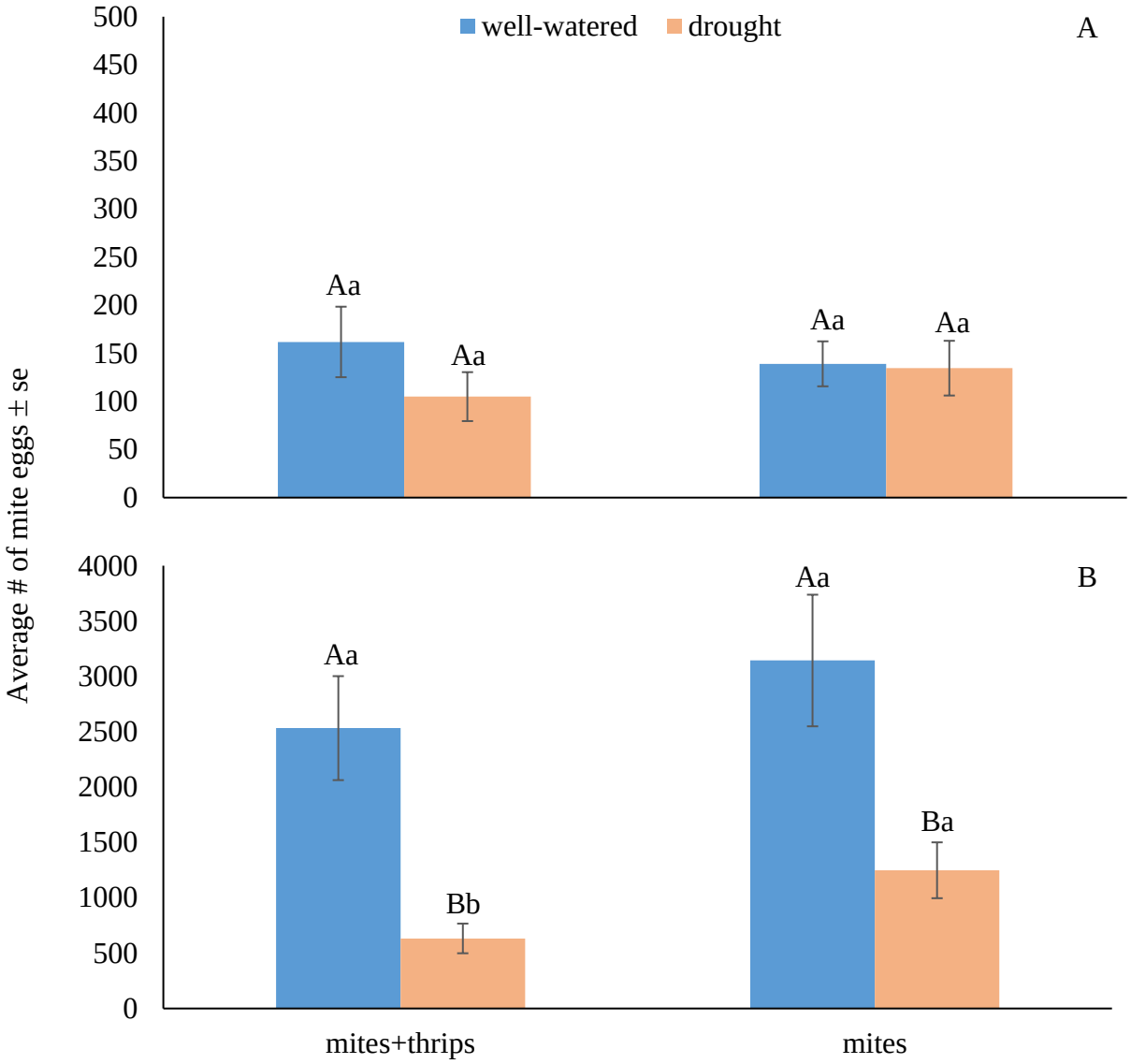

Figure 2.11 Mite egg populations of well-watered and drought stressed plants with and without thrips at two different sample dates: (A) *Sample 1* and (B) *Sample 2*. Uppercase letters assigned to data bars signify a within-arthropod treatment comparison and lowercase letters signify an across-arthropod treatment comparison. Significance set at $p<0.05$.

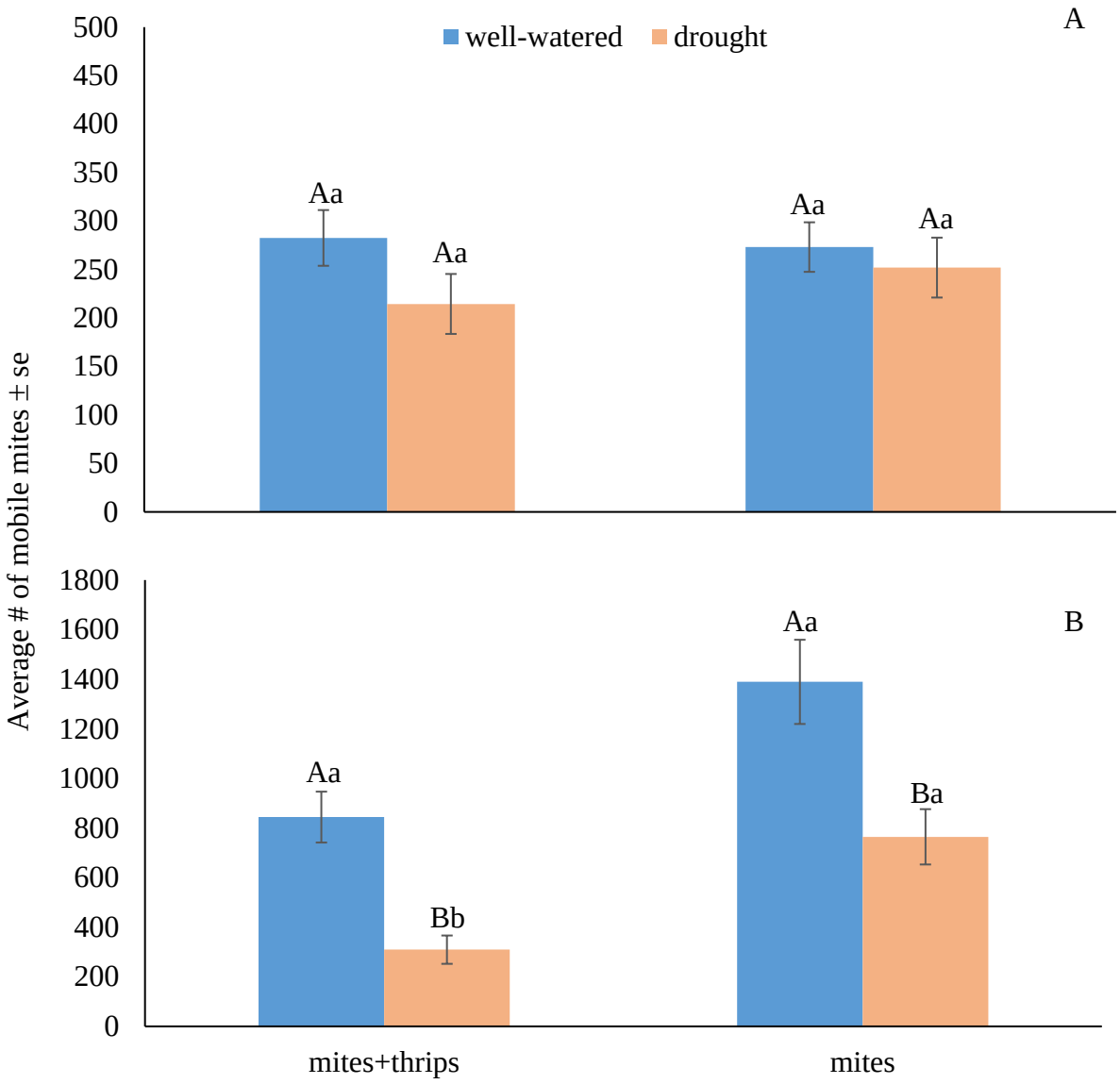

Figure 2.12 Mobile mite populations of well-watered and drought stressed plants with and without thrips at two different sample dates: (A) *Sample 1* and (B) *Sample 2*. Uppercase letters assigned to data bars signify a within-arthropod treatment comparison and lowercase letters signify an across-arthropod treatment comparison. Significance set at $p<0.05$.

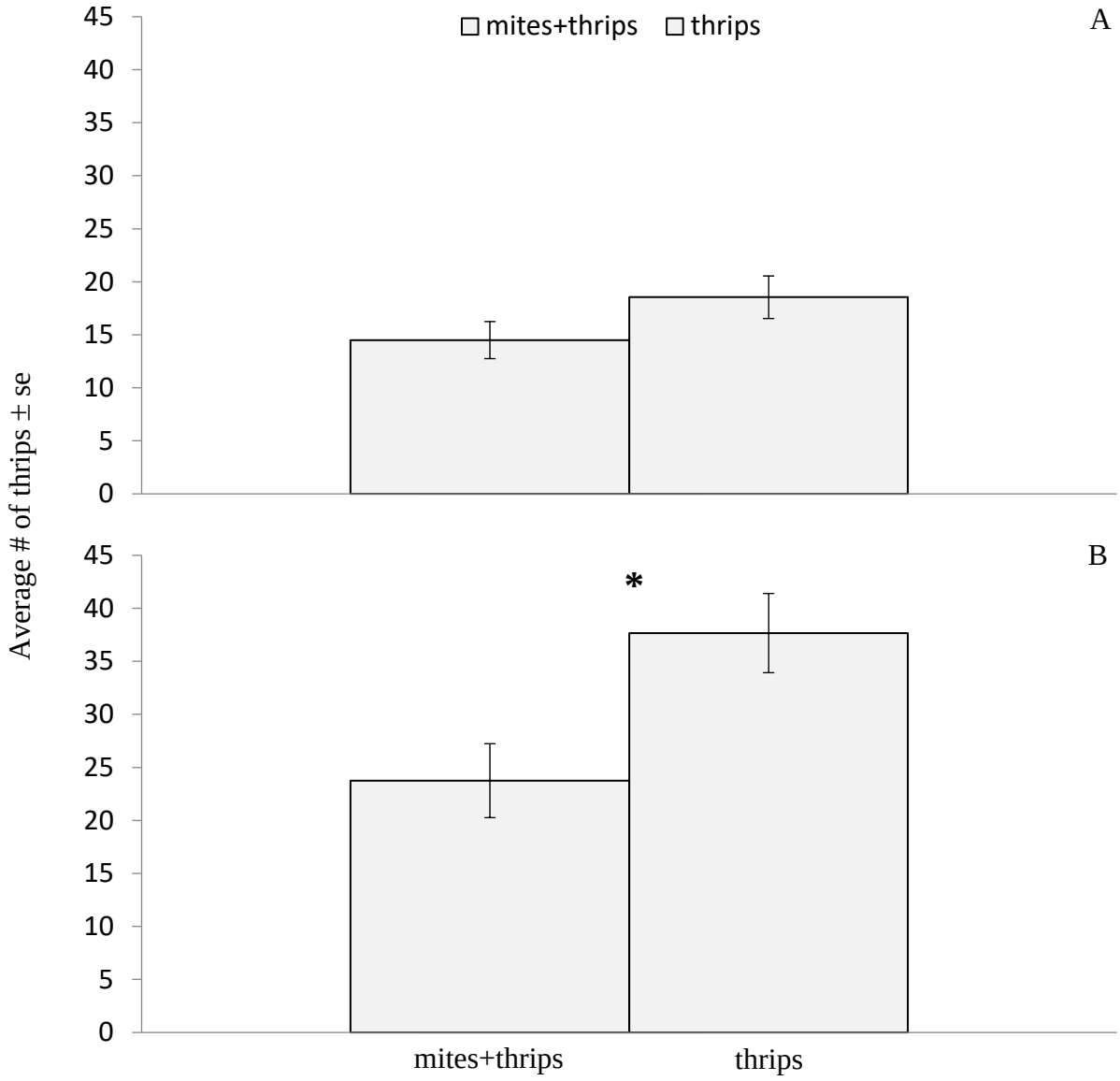

Figure 2.13 Thrips populations of plants with mites+thrips and only thrips at two different sample datess: (A) *Sample 1* and (B) *Sample 2*. Asterisk signifies statistical significance ($p<0.05$).

Table 2.1 Statistical analysis of plant and arthropod response variables.

analysis	type III tests of fixed effects	df	f	p
plant height (repeated measurement)	water	20	320	<0.0001
	mites	66	0.02	0.8951
	water*mites	66	0.3	0.588
	thrips	66	0.81	0.3727
	water*thrips	66	0.97	0.3278
	mites*thrips	66	0.3	0.588
	water*mites*thrips	66	0.1	0.7583
	sday	88	425.59	<0.0001
	water*sday	88	130.68	<0.0001
	mites*sday	88	3.87	0.0524
	water*mites*sday	88	1.07	0.3044
	thrips*sday	88	0.03	0.868
	water*thrips*sday	88	0.01	0.9206
	mites*thrips*sday	88	1.69	0.1971
	water*mites*thrips*sday	88	0.05	0.8161
nodes (repeated measurement)	water	20	250.78	<0.0001
	mites	66	3.19	0.0786
	water*mites	66	0.51	0.4774
	thrips	66	0.13	0.722
	water*thrips	66	0	1
	mites*thrips	66	3.19	0.0786
	water*mites*thrips	66	2.04	0.1577
	sday	88	257.19	<0.0001
	water*sday	88	14.71	0.0002
	mites*sday	88	4.38	0.0393
	water*mites*sday	88	1.09	0.2985
	thrips*sday	88	1.94	0.1667
	water*thrips*sday	88	0.12	0.7282
	mites*thrips*sday	88	0	1
	water*mites*thrips*sday	88	0.12	0.7282
stomatal resistance (repeated measurement)	water	20	59.61	<0.0001
	mites	66	1.44	0.2345
	water*mites	66	0	0.9639
	thrips	66	0.24	0.6255

	water*thrips	66	1.23	0.2706
	mites*thrips	66	8.73	0.0043
	water*mites*thrips	66	1.93	0.169
	sday	88	6.25	0.0143
	water*sday	88	0.43	0.5158
	mites*sday	88	2.31	0.1319
	water*mites*sday	88	1.99	0.1615
	thrips*sday	88	5.13	0.0259
	water*thrips*sday	88	2.75	0.1008
	mites*thrips*sday	88	0	0.9928
	water*mites*thrips*sday	88	0.03	0.8731
leaf temp °C (repeated measurement)	water	11	0.83	0.3807
	mites	66	1.53	0.2199
	water*mites	66	1.05	0.3086
	thrips	66	1.05	0.3086
	water*thrips	66	1.23	0.2714
	mites*thrips	66	0.02	0.8974
	water*mites*thrips	66	1.13	0.2916
	sday	88	21.03	<0.0001
	water*sday	88	0.38	0.5386
	mites*sday	88	0.6	0.4388
	water*mites*sday	88	0.59	0.4436
	thrips*sday	88	0.23	0.6317
	water*thrips*sday	88	0.46	0.4974
	mites*thrips*sday	88	1.79	0.184
	water*mites*thrips*sday	88	0.45	0.5024
leaf relative humidity (repeated measurement)	water	11	80.27	<0.0001
	mites	66	1.58	0.2139
	water*mites	66	1.56	0.2155
	thrips	66	0.01	0.9228
	water*thrips	66	1.35	0.2502
	mites*thrips	66	12.4	0.0008
	water*mites*thrips	66	0.63	0.4302
	sday	88	1.73	0.1922
	water*sday	88	0.15	0.6974
	mites*sday	88	0.85	0.3588
	water*mites*sday	88	1.55	0.2158

	thrips*sday	88	4.97	0.0284
	water*thrips*sday	88	1.12	0.2919
	mites*thrips*sday	88	0.01	0.9137
	water*mites*thrips*sday	88	1.1	0.2975
plant height (non-repeated, *pre-arthro*)	water	11	59.36	<0.0001
nodes (non-repeated, *pre-arthro*)	water	7	57.17	<0.0001
stomatal resistance (non-repeated, *pre-arthro*)	water	20	117.22	<0.0001
leaf temp °C (non-repeated, *pre-arthro*)	water	177	0.13	0.7147
leaf relative humidity (non-repeated, *pre-arthro*)	water	20	89.7	<0.0001
plant height (non-repeated, *post-arthro1*)	water	20	319.25	<0.0001
	arthro	157	0.56	0.6451
	water*arthro	157	1.11	0.345
nodes (non-repeated, *post-arthro1*)	water	11	541.31	<0.0001
	arthro	160	0.39	0.7592
	water*arthro	160	0.12	0.949
stomatal resistance (non-repeated, *post-arthro1*)	water	20	69	<0.0001
	arthro	160	2.49	0.0623
	water*arthro	160	2.01	0.1144
leaf temp °C (non-repeated, *post-arthro1*)	water	171	0.52	0.4718
	arthro	171	14.42	<0.0001
	water*arthro	171	0.16	0.9233
leaf relative humidity (non-repeated, *post-arthro1*)	water	20	94.23	<0.0001
	arthro	160	1.74	0.1606
	water*arthro	160	1.37	0.2539
plant height (non-repeated, *post-arthro2*)	water	20	260.78	<0.0001
	arthro	66	0.35	0.7857
	water*arthro	66	0.27	0.85
nodes (non-repeated, *post-arthro2*)	water	20	190.56	<0.0001
	arthro	66	3.27	0.0265
	water*arthro	66	0.57	0.6367
stomatal resistance (non-repeated, *post-arthro2*)	water	22	62.82	<0.0001
	arthro	66	3.74	0.0152
	water*arthro	66	2.01	0.1213
leaf temp °C (non-repeated, *post-arthro2*)	water	77	0.3	0.5831
	arthro	77	5.24	0.0024
	water*arthro	77	0.46	0.7099
leaf relative humidity (non-repeated, *post-arthro2*)	water	11	56.59	<0.0001

	arthro	66	5.72	0.0015
	water*arthro	66	1.69	0.1786
root length	water	174	2.15	0.1448
	arthro	174	0.21	0.8914
	water*arthro	174	0.23	0.8772
	sday	174	2.15	0.1448
	water*sday	174	0.17	0.6843
	arthro*sday	174	0.97	0.4077
	water*arthro*sday	174	1.34	0.2626
total # of mites	trial	86	0.54	0.5862
	water	86	28.39	<0.0001
	arthro	86	5.41	0.0224
	water*arthro	86	1.57	0.2138
	sday	86	168.33	<0.0001
	water*sday	86	14.16	0.0003
	arthro*sday	86	2.88	0.0932
	water*arthro*sday	86	0.02	0.8816
# of mite eggs	trial	86	1.38	0.2568
	water	86	27.03	<0.0001
	arthro	86	3.4	0.0686
	water*arthro	86	2.07	0.1543
	sday	86	295.22	<0.0001
	water*sday	86	12.78	0.0006
	arthro*sday	86	1.8	0.1835
	water*arthro*sday	86	0.01	0.9246
# of mobile mites	trial	86	12.9	<0.0001
	water	86	34.57	<0.0001
	arthro	86	18.78	<0.0001
	water*arthro	86	2.15	0.1461
	sday	86	137.9	<0.0001
	water*sday	86	15.72	0.0002
	arthro*sday	86	16.77	<0.0001
	water*arthro*sday	86	0.46	0.5008
total # of thrips	trial	84	2.19	0.1185
	water	84	0.52	0.4748
	arthro	84	8.19	0.0053
	water*arthro	84	0.79	0.3779

	sday	84	21.53	<0.0001
	water*sday	84	0.17	0.6794
	arthro*sday	84	0.38	0.5393
	water*arthro*sday	84	1.45	0.2314
thrips scars	trial	84	65.93	<0.0001
	water	84	0.27	0.6032
	arthro	84	0.6	0.4425
	water*arthro	84	0.05	0.818
	sday	84	45.78	<0.0001
	water*sday	84	0.43	0.5158
	arthro*sday	84	1.36	0.2464
	water*arthro*sday	84	0.26	0.6135

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Chapter 3 - Western flower thrips predation of twospotted spider mite eggs increases in response to drought

Introduction

Crop production in the High Plains region of the United States is facing challenges due to the depletion of the underground water resource, the Ogallala Aquifer. Farm operations do not function in this region without aquifer irrigation, and they have a value of $3 billion annually (Garcia et al. 2018). Overexploitation is causing water-level reductions that the annual recharge rate cannot replenish (Scanlon et al. 2012). As a result of climate change, extended periods of drought between rain events are expected to become the norm by the year 2080, increasing irrigation rates, which will add additional stress on the aquifer (Karl et al. 2009). Preparation for hotter and drier conditions requires that we understand crop pest behaviors so that proper management strategies are readily available.

In a drought setting, pests may alter their diet in response to the environmental conditions and the physiological and biochemical changes of their host plant. Such an example is found in the arthropod pest communities of cotton. Although western flower thrips (*Frankliniella occidentalis*) are herbivores, they have been known to opportunistically feed on the eggs of twospotted spider mite (*Tetranychus urticae)*, a coexisting pest of early season cotton (*Gossypium hirsutum*). This host-sharing provides an opportunity for thrips to feed on both cotton tissue and spider mite eggs (Agrawal et al. 1999, Martini et al. 2013). The consumption of alternative food sources, such as the mites, may benefit thrips by allowing them to acquire nutrients that the plant cannot sufficiently provide under stress (Coll and Guershon 2002, Trichilo and Leigh 1988). The likelihood that thrips will feed on available, alternative food items has been correlated with decreasing quality of the plant that is available (Trichilo and Leigh 1988, Janssen et al. 2003). Drought could induce changes in the plant quality in a way that shifts

the food choice of thrips. Our objective was to examine the influence of drought on the omnivorous feeding behavior of thrips during early plant development. We predicted that thrips experiencing drought conditions would feed more on spider mites and less on plant material. To achieve our objective, we quantified western flower thrips' consumption of cotton leaf tissue and twospotted spider mite eggs while inhabiting either drought-stressed or well-watered cotton seedlings.

Materials and Methods

Mites colonies were collected from field corn (*Zea mays*) in Garden City, Kansas and subsequently reared on cotton seedling in a growth chamber at 27°C, with 16-hr light:8-hr dark.

Thrips used in the experiment originated from soybeans (*Glycine max*) from Garden City, Kansas and reared in the laboratory on green beans (*Phaseolus vulgaris*) in 15 cm by 15 cm plastic containers. A 7-cm hole in the lid of the container was fixed with polyester mesh (120 micron- open area %:34, mesh No.07-120/34; ELKO Filtering Company Tamarac, Florida) to allow airflow, retain thrips, and exclude unwanted arthropods. Artificial metal halide lighting was set to a 16-hr light:8-hr dark photoperiod and room temperature averaged approximately 24°C.

Cotton seeds (Bayer '121353', insecticide-free seed) were sown in air-dried potting mix (SunGro Sunshine® VP Metro-Mix® 250, Sun Gro Horticulture, Agawam, MA) in 10-cm diameter by 10-cm tall pots with a moistened coffee filter at the bottom to retain the potting mix. Cotton plants were thinned back to a single plant per pot after germination. Cotton plants were placed randomly within a growth chamber under artificial lighting (14-hr light:10-hr dark, 24°C, 80% relative humidity) until the first true-leaf appeared. Fertilizer water (20-10-20, N-P-K,

dissolved crystalized) was then added until all pots reached 100% saturation and the weight was recorded. This is what we call the original weight at saturation. During cultivation, all pots were weighed every other day to determine when to irrigate and the amount of irrigation applied to the pots differed according to the assignment of the water treatment. Well-watered plants were watered back to the original weight at saturation every other day. Drought stressed plants went without irrigation until they dropped below 30% of the original weight at saturation, at which point they were given enough water to reach 45% of the original weight at irrigation. Despite this difference in water supply, all plants developed to the first true-leaf stage ~24 days after planting. No irrigation water was added for the remainder of the experiment beyond this point.

The experiment was set up as a 2x2 factorial design with two watering regimes (drought stressed [D] or well-watered [W]) and two mite egg levels (present [TE] or absent [T]). Thrips were present in all treatments and there were the number of replications were as follows: D-T = 20 reps; D-TE = 23 reps ; W-T = 20 reps; W-TE = 18 reps. For each replication and treatment, a plastic exclusion cage (10 cm diameter x 8 cm tall) was placed around the first true-leaf of the seedling and attached to the petiole with modeling clay to create a seal (Figure 3.1). The cage was given ventilation holes sealed with 120-micron mesh to allow air flow. Once cages were attached, 30 adult female mites were placed on the first true-leaf of plants in the treatments with mites. After 24 hours, the adult mites were removed and eggs were counted. Excess eggs were removed using a soft brush so that exactly 120 eggs were left. Then, fifteen first-instar thrips were placed on the leaf using a small leaf disc.

Seventy-two hours after inoculation with thrips, the first true-leaf was removed from the stem and photographed using a digital camera. Using these photographs, the total surface area of scarred plant tissue caused by the feeding of thrips was quantified using ImageJ software (United

States National Institutes of Health, Bethesda, MD). Consumed eggs were quantified by subtracting the number of unconsumed eggs that remained on the leaf from the original 120 egg cohort. No immature mites were recovered during data collection, meaning that no eggs hatched at any point during the experiment.

Statistical analysis

Thrips leaf scar data were pooled and analyzed using PROC MIXED (allows the data to exhibit correlation and nonconstant variability), unequal variances analysis as a generalized linear model with Kenward-Roger degrees of freedom approximation in SAS 9.4. All combinations of water and arthropod treatments were compared for LSM differences. Egg consumption data were pooled and analyzed using PROC GLIMMIX (allows the data to exhibit correlation and nonconstant variability) analysis as a generalized linear model in SAS 9.4. Water treatments were compared for LSM differences. Alpha significance level was 0.05 for all analyses.

Results

Western flower thrips nymphs consumed twice as many mite eggs on drought-stressed cotton seedlings than on well-watered seedlings ($P = 0.006$, $F = 8.39$, $df = 1,39$) (Figure 3.2). However, neither water stress ($p = 0.08$, $F = 2.52$, $df = 1,88$) nor the presence of mite eggs ($p = 0.11$, $F = 2.27$, $df = 1,78$) significantly affected the amount of thrips scars on plant tissue after three days.

Discussion

On both the drought and well-watered plants, thrips appeared to exhibit the same level of plant feeding. However, consumption of mite eggs was greater on plants that experienced drought stress. It is unclear why adult thrips increased egg predation on drought-stressed plants,

but they may have been compensating for water loss. For example, other studies have shown that plants that are consistently drought stressed have lower turgor pressure and hardened tissue (Verslues 2005, Cutler 1978). Additional water loss from thrips due to respiratory and metabolic processes may have driven thrips to search for additional sources of water from food that require less energy to consume. Mite eggs are immobile and succulent, which helps explain the consumption of nearby mite eggs. As mite populations grow, these eggs would not be as easily accessible, as they were in our study, due to the buildup of mite webbing, which can inhibit thrips foraging activity (Trichilo & Leigh 1986). However, thrips would likely continue to consume mite eggs when encountered, because supplementing their water intake by adding mite eggs to their diet quickens young instar develop, and those that reach adulthood are more fecund and survive longer (Trichilo and Leigh 1988).

An alternative explanation for higher predation rates by thrips in a drought setting is the induction of host plant defenses to herbivory. Agrawal et al. (1999) showed that the plant defense compound, jasmonic acid (JA), is released by arthropod herbivory and influenced by drought stress via abscisic acid (Erb et al. 2012). Under well-watered conditions, JA induction via spider mite feeding damage is known to cause thrips to consume more spider mites (Li et al. 2002). With higher defenses from the host plant, the thrips may utilize spider mite eggs to supplement their diet. The fact that we recorded the same thrips scars between water treatments means that thrips continued to feed on cotton tissue and suggests that cotton was a suitable food source. We expected thrips to feed more on drought stressed plants than well-watered plants if the nutritional quality of plant tissue of drought-stressed plants was lower. However, we did not analyze the nutritional status of cotton seedlings under different levels of water stress.

From a pest management perspective, the increased consumption of spider mite eggs while retaining the same amount of leaf tissue has positive implications for pest management. Under such a scenario when the crop is facing environmental stress from drought, the removal of spider mite eggs would result in fewer total pests in the system. This study encourages the development of new action thresholds of thrips and mites in the High Plains. Current action thresholds for thrips is one thrips per true leaf, and our study does not support changing this threshold under drought conditions because of the continued potential for yield-damaging deformation that thrips can cause by feeding while new seedling leaves are emerging. Further research needs to examine better biological control efforts during these adverse conditions to reduce economic and environmental costs of pesticide applications.

Figures

Figure 3.1 Exclusion cage containing cotton leaf and arthropods. Modeling clay was used to create a tight seal where the cage wrapped around the leaf petiole. Fine mesh was used on the ceiling of the plastic cage. Green paper cups and wooden sticks were used solely as structural support.

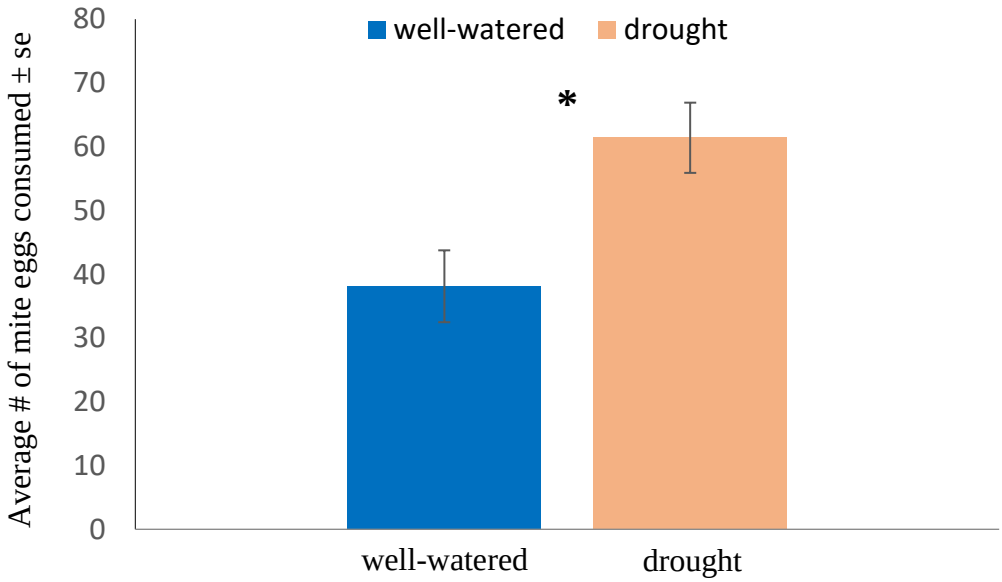

Figure 3.2 The number of mite eggs consumed by thrips on well-watered and drought stressed plants after 72 hours. Asterisk signifies statistical significance ($p<0.05$).

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Chapter 4 - Summary and Future Directions

Based on our greenhouse and laboratory experiments, it is likely that an increase in the frequency of drought conditions on the High Plains, as predicted by climate change models, would have both direct and indirect effects on cotton. Water stress had predictable negative effects on several plant responses. In addition, drought conditions in cotton seedlings created indirect tri-trophic, or three-way food web, effects impacting twospotted spider mites and western flower thrips at the second trophic level (herbivores), and western flower thrips at the third trophic level (facultative predators). Our experiments showed that seedling plants experienced a compounded net effects from drought because of the plant-damaging feeding activity of pests during our experiments, and because plant performance was negatively impacted by drought. As such, our results suggest that early-season crop management strategies would benefit from certain alterations. Specifically, High Plains producers would benefit from selecting cotton varieties that have higher drought-tolerant traits because of their ability to develop and mature under low-water settings. Additionally, it is unlikely that producers would benefit from having thrips in their seedling cotton fields because the beneficial predation of mite eggs does not offset the detrimental damage that they have on the developing young leaves. Further research into the immediate biological control of thrips and mites in seedling cotton experiencing drought stress is needed if farmers are to reduce their costly applications of pesticides. Such areas of focus would be proximity to habitat that harbors beneficial arthropods, and interactions between a diverse beneficial arthropod community on these small plant hosts.

Findings from the greenhouse experiment (Chapter 2) suggest that drought conditions during the seedling stage may enhance the suppression of mites and thrips via competition. The

degree of drought that we imposed was tolerated by the cotton seedlings, and with the decrease of total arthropod populations when exposed to drought conditions (decline in mites and no change in thrips) the plant was shown to withstand drought despite the presence of pests. Longer-term experiments and experiments that change the severity of water availability would bolster our understanding of these early-season interactions between plant and arthropods. Continued research could record cotton fiber yield to help understand the long-term impacts from early season drought. A balance between water conservation and pest management will allow producers to reduce operational costs and ensure a stable agricultural economy in the High Plains and elsewhere.

The laboratory experiment (Chapter 3) designed to test whether drought-stressed plants caused a shift in thrips feeding behavior towards greater predation on spider mite eggs helped to explain reductions in mite populations observed in the greenhouse experiment by documenting that adult thrips increased their consumption of mite eggs under drought conditions. Although the level of plant feeding did not decrease as we had predicted based on the assumption that drought would lower plant quality for thrips, we hypothesized that the increase in egg predation may have been a means of acquiring additional water. Interestingly, when no mites were available, we did not observe an increase in plant feeding under drought conditions, which could have compensated for reduced water content in cotton leaves. To test this hypothesis, future experiments could compare wet versus dry body weights of thrips under different levels of water treatments. Other ways to approach this would be to measure thrips reproduction and survival, also known as fitness, under different water settings which would give insight into the nutritional qualities of water to drought stressed thrips.

Understanding the interactions between plant and arthropods under various environmental conditions can provide a further level of preparedness under a changing climate and water resource management. We hope that the impact of the population level study of Chapter 2 and the behavioral level study of Chapter 3 will help guide future research questions, crop pest management decisions, and irrigation regimes that will prepare the agricultural industry for dry conditions during the growing season.

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